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Effectiveness and satisfaction of dietary supplements investigation 2021

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Abstract

Obesity is a serious cause of most maladies. Health-conscious people are aware of their weight. Individuals who can control their weight and body mass index (BMI) will remain vigorous life expectancy. Objectives: 1. To compare weight and BMI loss of a randomly selected dietary supplements food for one time/day in 30 days. 2.To verify consumer's satisfaction of the dietary supplements selected. Method: A cross-sectional true experimental design was performed in July 2021. Among 47 dietary supplements food in Thailand market, only one brand was randomly selected. Researchers contacted and submitted research proposal to the company. The company accepted this challenge and supplied only product for this investigation. Population were Thai age between 21-60, from Bangkok and the central part of Thailand. Sample was calculated via Jacob Cohen (1977). Once set α=0.05, β=0.35, Power=0.80, Effect size=0.40 yielded sample size (n)=34. SPSS (version 17) was employed to evaluate all data. Results: All 34 (100%) respondents, 19 (55.90%) were female and 15 (44.10%) were male, The average age was 32.03±5.79 years, average height was 165.02±6.32 cm, average weight was 69.71±7.72 kg, average BMI was 24.47±2.39 km/m^2. Eating and exercise behavior were 2 serious extraneous variables to be controlled in this inspection, however, when tested eating and exercise behavior did not significantly change when measured on day 1 and day 30 (p=0.180 and 0.688, respectively McNemar test). Paired t-test was employed to compare means of weight and BMI on day 1 and day 30. It confirmed that the average weight and BMI were significantly decreased (p=0.000**and 0.000**, paired t-test respectively). Moreover, the average satisfaction on taste, smell, dissolution rate, packaging and satisfaction on weight reduction were 4.57±0.51, 4.47±0.71, 4.57±0.46, 4.77±0.83 and 4.69±0.74 respectively. Conclusion: Thai Dietary supplements 1 time/day for 30 days could significantly reduce weight and BMI. Participants were satisfied with the product.

Keywords: Obesity; Dietary supplements food; Experimental design; Paired t-test

1. Introduction

A major source of health concern in Thailand is obesity (Jiranin et al, 2011) with 32% of the population identifying as overweight and 9% obese. According to World Health Organization Thailand has one of the highest incidence of overweight citizens in South-East Asia Region (WHO, 2019) The Thai National Health Examination Surveys (NHES) found that obesity in Thailand more than doubled during the period 1991-2014 (Aekplakorn et al, 2014). This was caused by increasing access to junk food

(Baker, P; Friel, 2014) and unhealthy switches from active to sedentary lifestyles (Teerawattananon and Luz, 2017). The prevalence of obesity in Thailand coincides with increased intake of sugary drinks. In 2010, it was reported that an average Thai consumed 93.9 litres of sweetened drinks on a yearly basis. This figure rose by 23.8% in 2015, to 115.6 litres (Euromonitor International; 2016.). The most important strategies for preventing obesity are healthy eating behaviors, regular physical activity, and reduced sedentary activity (such as watching television and videotapes, and playing computer games).

Three major ways to reduce weight are; increase exercise activities, decrease over-eating behavior and intake dietary supplement food for dinner. Dietary supplements include such ingredients as vitamins, minerals, herbs, amino acids, and enzymes. Dietary supplements are marketed in forms such as tablets, capsules, softgels, gelcaps, powders, and liquids. One of the few accepted dietary supplements food of choices is whey protein.

Whey protein is one of the two proteins found in cow's milk. When milk is used to produce cheese, the curding process separates the curds from the liquid, also known as whey. Whey is a nutrient-dense, nearly translucent liquid consisting of all nine essential amino acids (the building blocks of protein) and vitamins and minerals. The liquid whey is filtered and dried to make whey protein powder (Lee, 2014).

Protein intake is important to maximize muscular function during resistance exercise. Based on the reports of the Food and Nutrition Board (FNB) of the Institute of Medicine, the Dietary Reference Intakes (DRIs) is 56 g/day for men aged 19–29 years. However, this is not sufficient to have a positive effect on the intracellular essential amino acid supply, prevent protein oxidation, recover muscle damage during exercise, increase muscle mass and strength (Kim et al, 2010). Therefore, protein supplementation is required to maximize the adaptive response of the skeletal muscle to prolonged resistance exercise training (RET). On the other hand, the net muscle protein balance remains negative in the absence of nutrient intake, indicating that the muscle tissue remains in a catabolic state, breaking down or losing overall mass, both fat and muscle (Pitkanen et al, 2003).

Objectives: 1. To compare weight and BMI loss of a randomly selected dietary supplements food for one time/day in 30 days. 2.To verify consumer's satisfaction of the dietary supplements selected.

2. Materials and Methods

A cross-sectional, prospective, true experimental research design was conducted during July 2021. This study was approved by Burapha University Ethics Committee on June 2021.

2.1 Dietary supplements food selection processes

Firstly, five nutrition scientist experts were agreed to set criteria to review and investigate the profile of all 87 dietary supplements food in the Thai market. Secondly, Criteria were 1. the real existing address of the company that could be interconnected and the people of the company were willing to provide information and scientific evidences about product. 2. The product must be verified by Thai Food and Drug Administration. 3. The main ingredients must be Wey protein and Carboxymethyl Cellulose (CMC). Only 47 qualified brands from 47 companies were qualified to be chosen to this study. Thirdly, only one brand from 47 eligible dietary supplements food was randomly selected to the experiment. Lastly, researchers contacted the chosen company and presented the protocol of this study. The first companies turned down the project however, the next second company accepted this challenged experiment and agreed to support dietary supplements food thorough this experiment.

2.2 Participants selection process

1. Population were Thai, age between 20-60, from Bangkok and the central part of Thailand. Since paired t-test was used to compared means of weight, BMI and satisfaction of product 4 times (before and after treatments) therefore, sample size was calculated via Jacob Cohen's table 1 page 384 (1977). Once set α=0.05, β=0.35, Power=0.80, Effect size=0.40 yielded sample size (n)=34.

2. Study protocol and volunteer recruitment procedure were announced on website. Among 115 applications, 34 were randomly selected to participate in this program.

3. All 34 chosen participants must join the group line. Dietary supplements food was directly sent by mail to participants every week for 4 weeks. Participants must take dietary supplements food for dinner without having dinner every day for 30 days. Each one must measure and report his/her weight, height and answer questions in the questionnaire on the 1st, 7th, 14th, 21st and 30th day of consuming dietary supplements food then send them to researcher by line. Whilst taking dietary supplements food, participants must take pictures and video clips, then send them to researchers by line as well.

The two major extraneous variables in this study were eating and exercise behavior. These two confounders were controlled by investigating the eating behavior and exercise behavior on day 1 and day 30. If these two covariates could be statistically proved that they were not significantly change, then the two confounders were controlled. Researchers could be more certain that changing in weight and BMI of the participants must depend only on dietary supplements food2.

2.3 Instrument:

The self-administrative questionnaire was consisted of 15 questions divided into four parts. 1. Demographic data; weight, height, gender, age, occupation and marital status 2. Satisfaction on dietary supplements food-a 5 dimensions, 10 measurement variables, 5 choices Likert scale were employed 3. Eating behavior and 4. Exercise behavior were measured in ordinal scales.

The collected data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 17.0. Demographic characteristics were summarized using descriptive statistics. The nominal or ordinal scale variables were expressed in frequencies and percentages whereas the ratio scale results were presents in mean ± standard deviation. An association between related ordinal variables used McNemar test, Parametric (paired t-test) were performed to compare means before and after. treatments. A $p<0.05$ was considered statistically significant in all directional (one-tailed) tests.

3. Results and Discussion

Final return rate was 34 (100.00 %). Consistency of product satisfaction scales was assessed for internal reliability with Cronbach's Alpha coefficient. The reliability coefficient was 0.82.

3.1 Demographic Characteristics of samples

Most participants were female 19 (55.88%), male 15 (44.12%), employees 29 (85.29%), students 5 (14.71%), married 22 (64.71%) and single 12 (35.29%). (Table 1).

Table 1 Demographic data (nonmetric).

Nonmetric variables	Attributes	Frequency	Percent
Gender	Female	19	55.88
	Male	15	41.12
Occupation	Employees	29	85.29
	Students	5	14.71
Marital status	Married	22	64.71
	Single	12	35.29

On day 1 of the experimental program, the sample average age was 32.03±5.79. Average weight was 69.71±17.72 kg. Average height was 165.02±6.32 cm. Average BMI was 24.47±2.39 kg/meter^2 (Table 2).

Table 2 Demographic data on day 1 (metric).

Metric variables	Mean±SD
Age	32.03±5.79
Weight	69.71±7.72
Height	165.02±6.32
Body Mass Index	24.47±2.39

Figure 1 Eating behavior of samples were measured on day 1 and day 30.

Eating behavior of samples were measured on day 1 and day 30. On day 1, heavy eating behavior was 17 (50.00%) and moderate eating behavior was 17 (50.00%), on day 30 heavy eating behavior was 12 (35.29%) and moderate eating behavior was 22 (64.71%). Eating behavior of participants were not significantly different between day 1 and day 30 ($p \geq 0.05$, *McNemar test*).

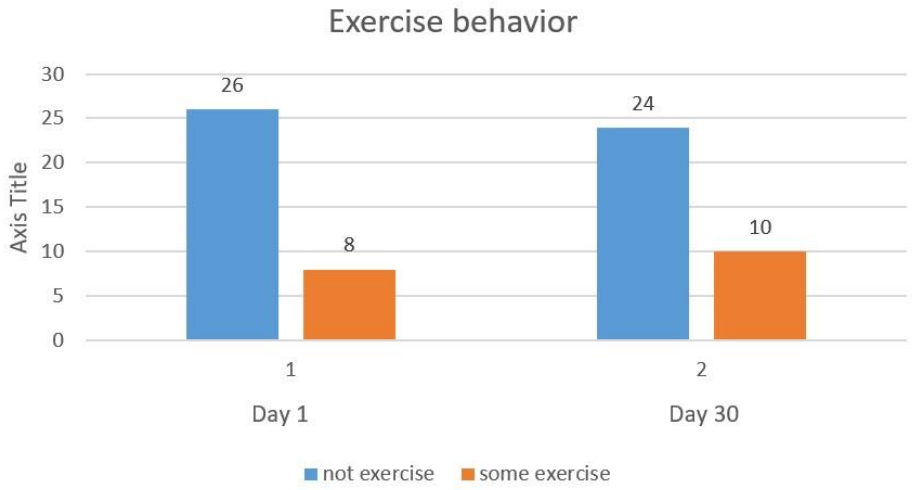


Figure 2 Exercise behavior of samples were measured on day 1 and day 30.

On day 1, not exercise was 26 (76.47%) and moderate exercise was 8 (23.53%), On day 30, not exercise was 24 (70.59%) and moderate exercise was 10 (29.41%). Exercise behavior of participants were not significantly different between day 1 and day 30 ($p \geq 0.05$, *McNemar test*).

McNemar test confirmed that eating behavior and exercise behavior did not change during the experiment then the two major extraneous variables were controlled.

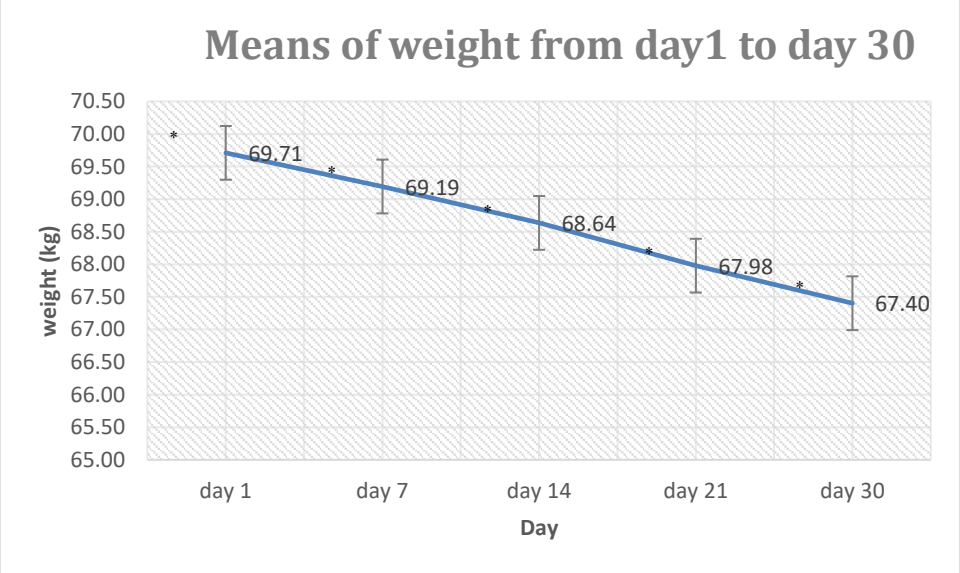
Paired t-test confirmed the significance weight reduction between day 1 and day 7, day 7 and day 14, day 14 and day 21, day 21 and day 30, day 1 and day 30 ($p < 0.05$, $p < 0.05$, $p < 0.05$, $p < 0.05$, $p < 0.05$, respectively, paired t-test) (Table 3).

Table 3 The mean±SD of weight and Body Mass Index on day 1, 7, 14, 21 and 30.

Day	Weight Mean±SD	P-value	BMI Mean±SD	P-value
1	69.71±7.72		24.47±2.66	
7	69.19±7.74[a]	$p<0.05$	24.36±2.67[e]	$p<0.05$
14	68.64±7.78[b]	$p<0.05$	24.09±2.47[f]	$p<0.05$
21	67.98±7.83[c]	$p<0.05$	23.87±2.74[g]	$p<0.05$
30	67.40±7.81[d]	$p<0.05$	23.66±2.63[h]	$p<0.05$

[a] indicated the average weight of day 7 was significantly lower than day 1
[b] indicated the average weight of day 14 was significantly lower than day
[c] indicated the average weight of day 21 was significantly lower than day 14
[d] indicated the average weight of day 30 was significantly lower than day 21
[e] indicated the average Body Mass Index of day 7 was significantly lower than day 1
[f] indicated the average Body Mass Index of day 14 was significantly lower than day 7
[g] indicated the average Body Mass Index of day 21 was significantly lower than day 14
[h] indicated the average Body Mass Index of day 30 was significantly lower than day 21

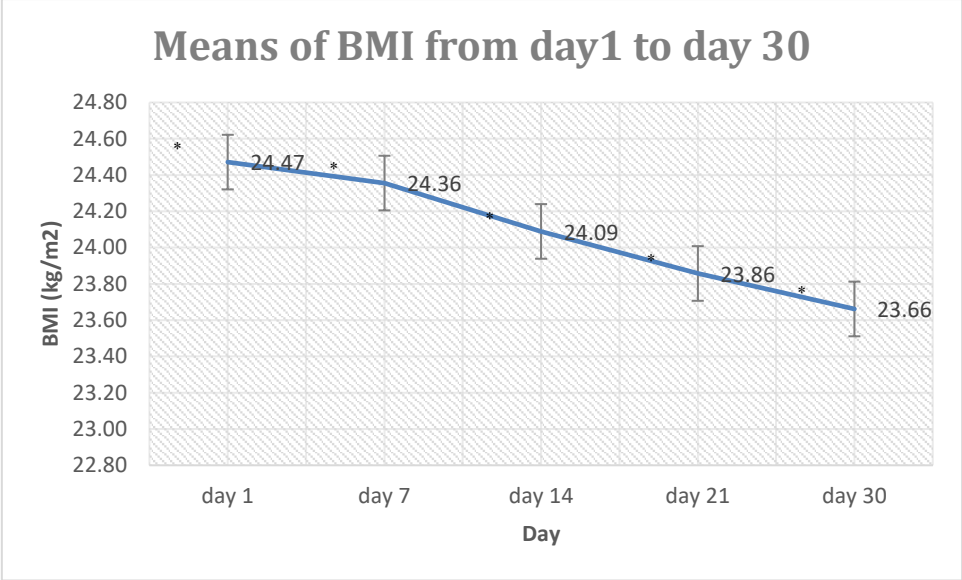
The average weight of participants on day 1 was 69.71 kg, day 7 was 69.19 kg, day 14 was 68.64 kg, day 21 was 67.98 kg and day 30 was 67.40 kg.



* $P<0.05$

Figure 3 Graph showed the average weight of participants measured on day 1, 7, 14, 21 and 30.

The average BMI of participants on day 1 was 24.47 Kg/m^2, day 7 was 24.36 Kg/m^2, day 14 was 24.09 Kg/m^2, day 21 was 23.86 Kg/m^2 and day 30 was 23.66 Kg/m^2.



* *P<0.05*

Figure 4 Graph showed the average BMI of participants measured on day 1, 7, 14, 21 and 30.

The survey revealed that product satisfactions were high in most aspects, namely taste was 4.57, smell was 4.47, dissolve was 4.57, package was 4.77 and reducing weight was 4.69 .

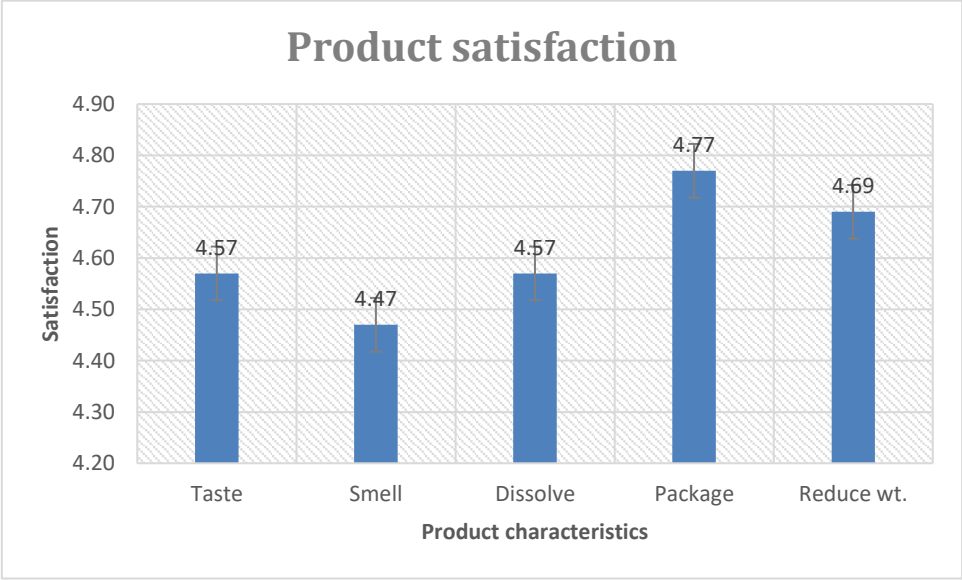


Figure 5 Product satisfaction scores

From the scale 5: taste was 4.57, smell was 4.47, dissolve was 4.57, packaging was 4.77 and feeling of weight reduction was 4.69.

4. Conclusion

This experiment confirmed that when eating behavior and exercise activities were controlled, the weight and BMI of randomly selected 34 participants were significantly reduced when took Thai dietary supplements food 1 time/day replace dinner for 30 days. The result of this study supported the pristine investigation by Machek et al (2019) and Roberfroid et al (2019). Moreover, this survey confirmed that participants were highly satisfied with the dietary supplement food.

5. Acknowledgements

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Comparison of effectiveness and satisfaction between Thai liniment and Singapore liniment

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Abstract

Liniment is a liquid or lotion, especially one made with oil, for rubbing on the body to relieve pain. Patients not only therapeutic effect desired, but also the quality of cosmetic grade of oil preferred. **Objectives:** to compare effectiveness and qualifications of Thai and Singapore liniments in 3 dimensions including: 1) Effectiveness i.e. onset and duration of pain relieving 2) Satisfaction i.e. color, viscosity, and heat sensation, 3) Consumer risk i.e. price, performance and side effect. **Method:** A true crossing over experimental design was performed to compare two liniment brands. Two sampling techniques were used: 1) Simple random sampling one Thai liniment brand and one Singapore liniment brand were chosen to be the representatives. 2) Burapha university student sport men from 10 kinds of sports were randomly selected. Sample size was calculated via Jacob Cohen's table 1978, with α=0.05, β=0.20, power=0.80 and effect size=0.40. Calculated sample size was n=26 in each group. Total sample size (N) was 52. The Visual Analogue Scale was used to measure pain relieving satisfaction into ratio scale. Paired t-test and ANOVA were employed to identify the significance of the mean differences. **Results**: The most favored pain relieved products dosage form among sport-men were sprays 7.38±1.06, creams 7.12±1.89, gels 6.41±1.37, patches 6.37±1.79, balms 6.02±1.51, liniments 5.97±1.08, and tablets 5.92±1.34.The mean differences of between effectiveness, satisfaction and consumer risk between Thai liniment brand and Singapore liniment brand were not significantly differences ($p \geq 0.05$) however the price of Thai liniment brand was significantly cheaper than Singapore liniment brand ($p<0.05$). **Conclusion:** We confirmed that Thai liniment brand and Singapore liniment brand liniment were not significantly different in all aspects namely, effectiveness, satisfaction and consumer risk except Thai price was cheaper. Therefore, Thai government should support Thai small industry likes the OTOP liniment products to compete and survive in international market.

Keywords: Liniment; Crossing-over; True experimental design; ANOVA, Paired t-test

1. Introduction

Liniment is a liquid or lotion, especially one made with oil, for rubbing on the body to relieve pain. Patients not only therapeutic effect desired, but also the quality of cosmetic grade of oil preferred for residual left on skin, counterirritant, color, smell, viscosity and side effect.

Liniment is a medicated topical preparation for application to the skin. Sometimes called a heat rub, a liniment may have viscosity similar to that of water, or may be formulated as a lotion or balm and are usually rubbed on skin to allow penetration of the active ingredients. Patches, sticks, and sprays are also available (De Belilovsky, Boyer, Bellemere, & Baudouin, 2020; DOWNING, 1946).

Liniments are usually sold to relieve pain, like from painful muscle aches and strains, or swelling. Liniments are normally formulated from alcohol, acetone, or similar rapidly-vaporizing solvents and contain counterirritant scented chemical compounds, such as methyl salicylate, benzoin resin, and menthol. They produce a sense of hotness within the muscle of the area where the liniments are applied. Sports liniments are typically viscous, and a gentle massage is necessary whereas applying sports liniment on the skin. Liniment gives fast relief to the stresses and muscular pain. Liniment is presented in diverse forms, such as compressed liquid, semi-solid, and liquid. Because of the fast-relieving ability to pain, sports liniment is expected to remain positive in the global market (Maximize Market Research, 2020; Stevenson, 2020).

2. Materials and Methods

2.1 Methods

A true crossing-over experimental design was performed to compare two liniment brands. This experiment was conducted during July to November 2018. The protocol of this study was approved by Burapha University Ethics Committee on June 2018.

2.2 Liniments selection processes

Firstly, three experts from Burapha University were agreed to set criteria to review and investigate the profile of all 36 Thai liniment and Singapore liniment brands in the Thai liniment market. Secondly, only 8 qualified Thai liniment brands and one Singapore brand were qualified to be chosen to this study. Thirdly, only one brand from 8 qualified Thai liniment brands was randomly selected to this experiment. Then a qualified Singapore liniment brand was selected. Finally, researchers contacted the chosen Thai liniment company and presented the protocol of the study. The company accepted the protocol and agreed to support its Thai liniment oil and also support the chosen Singapore liniment for this experiment.

2.3 Participants selection process

Population was all student athlete from 10 sports of The Burapha University in the year 2019. The selected sports were; football, basketball, volleyball, sepak-takro, boxing, swimming, running, badminton, tennis, and athlete.

Sample size was calculated via Jacob Cohen's table 1988 (Cohen, 1988). α was set at 0.05, β=0.20, power=0.80, effect size=0.40, yielded n=26 in each group. Total sample size (N) was 26*2=52. Probability simple random sampling was executed to select samples (sport-men students).

1. Fifty-two (52) sport men from 10 sports from Burapha University were randomly chosen to be samples in this experiment. 2. Samples were randomly assigned into two groups (A and B). 3. The two groups were randomly selected to be treatment group A (Thai liniment treatment first, then Singapore liniment treatment) and group B (Singapore liniment treatment first, then Thai liniment treatment).

Crossing-over experimental design was performed. Group A started using Thai liniment for 7 days and answered the questionnaire. Three days-washout period-was deferred to let body eliminate the effect of Thai liniment. Then started using Singapore liniment for 7 days and answered the questionnaire. Whereas, Group B started using Singapore liniment for 7 days and answered the questionnaire. Three days-washout period-was deferred to let body eliminate the effect of Singapore liniment. Then started using Thai liniment for 7 days and answered the questionnaire.

2.4 Instrument:

The self-administrative questionnaire was consisted of 19 questions divided into four parts. 1. Demographic data; age, kind of sports. 2. Effectiveness i.e., onset and duration of pain relief 3. Satisfaction i.e., color, smell, viscosity, residual left on skin and counterirritant effect, 4. Consumer risk i.e., price, performance and side effect. The Visual Analogue Scale was used to measure all three dimensions (10 measurement variables) namely; effectiveness, satisfaction and consumer risks in ratio

scale. The collected data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 17.0. Demographic characteristics were summarized using descriptive statistics. The nominal or ordinal scale variables were expressed in frequencies and percentages whereas the ratio scale results were presents in mean±standard deviation. parametric paired t-test was used to compare means before (treatment A-Thai liniment) and after (treatment B-Singapore liniment) of group A. and to compare means before (treatment B-Singapore liniment) and after (treatment A-Thai liniment) of group B. Whereas, one way ANOVA was used to compare means of the two independent groups (A and B, 2 times). A $p<0.05$ was considered statistically significant in all directional (one-tailed) tests (Figure 1).

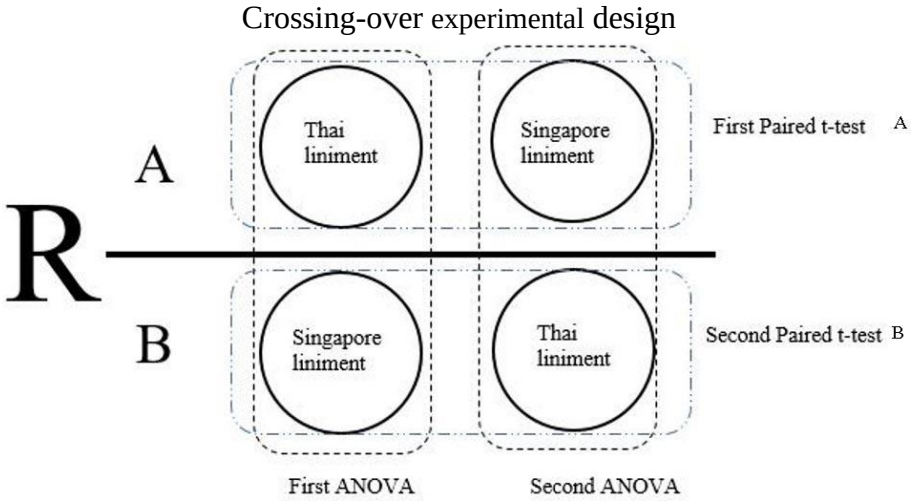

Figure 1 Crossing-over experimental design and plan to designate statistical procedures in this experiment. (Campbell and Stanley, 2015; Steven, 1986)

3. Results and Discussion

All 52 (100.00 %) samples were completely collected data. Most participants were male 41 (78.80%), female 11 (21.20%), group A (start using Thai liniment, then Singapore liniment) 26 (50.00%), B (start using Singapore liniment, then Thai liniment) 26 (50.00%), football 6 (11.50%), basketball 4 (7.70%), volleyball 4 (7.70%), sepak-takrow 4 (7.70%), boxing 6 (11.59%), swimming 6 (11.59%), running 6 (11.59%), badminton 6 (11.59%), tennis 6 (11.59%) and athlete 4 (7.70%) (Table 1).

Table 1 Demographic data (nonmetric variables).

Variables	Attributes	Frequency	Percent
Gender	Male	41	78.80
	Female	11	21.20
Groups	A (start using Thai liniment, then Singapore liniment)	26	50.00
	B (start using Singapore liniment, then Thai liniment)	26	50.00
Sports	Football	6	11.50
	Basketball	4	7.70
	Volleyball	4	7.70
	Sepak-takrow	4	7.70
	Boxing	6	11.50
	Swimming	6	11.50
	Running	6	11.50
	Badminton	6	11.50
	Tennis	6	11.50
	Athlete	4	7.70

The first ANOVA test was employed to compare means of 10 aspects between Thai and Singapore liniment. The results were; pain relief, onset and duration, color, smell, viscosity, residual, counterirritant, performance and side effect were not significantly different ($p \geq 0.05$), however only the price of Thai liniment was significantly cheaper than Singapore liniment ($p < 0.05$) (Table 2).

The second ANOVA test was employed to compare means of 10 aspects between Thai and Singapore liniment. The results were; pain relief, onset and duration, color, smell, viscosity, residual, counterirritant, performance and side effect were not significantly different ($p \geq 0.05$), however only the price of Thai liniment was significantly cheaper than Singapore liniment ($p < 0.05$) (Table 2).

Paired t-test A was executed to compare means of pain relief, onset and duration, color, smell, viscosity, residual, counterirritant, price, performance and side effect. There were not significantly different between Thai and Singapore liniment in all aspects ($p \geq 0.05$) (Table 3).

Paired t-test B was executed to compare means of pain relief, onset and duration, color, smell, viscosity, residual, counterirritant, price, performance and side effect. There were not significantly different between Thai and Singapore liniment in all aspects ($p \geq 0.05$) (Table 4).

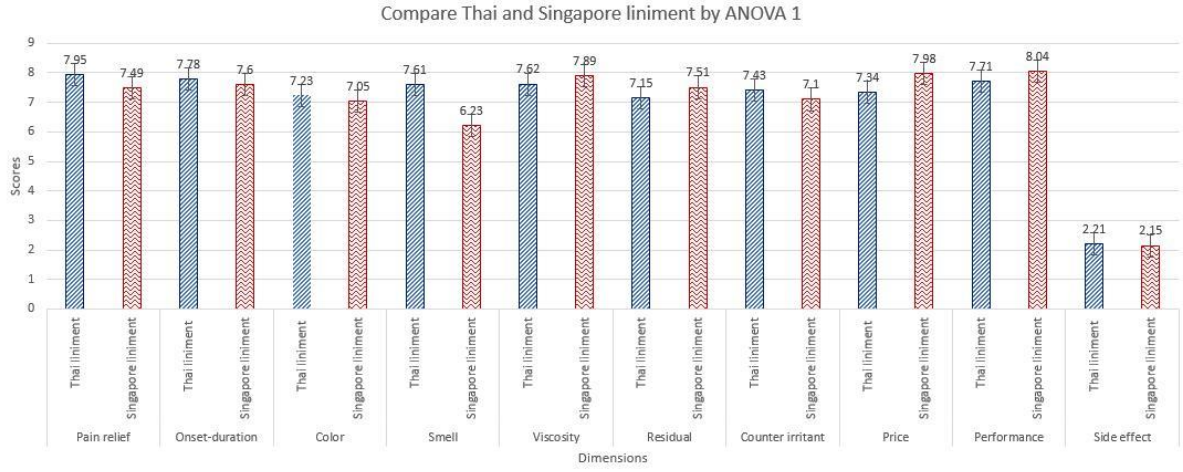

Figure 1 Compare means by the first ANOVA of Thai and Singapore liniment in 10 aspects namely; pain relief, onset and duration, color, smell, viscosity, residual, counterirritant, price, performance and side effect.

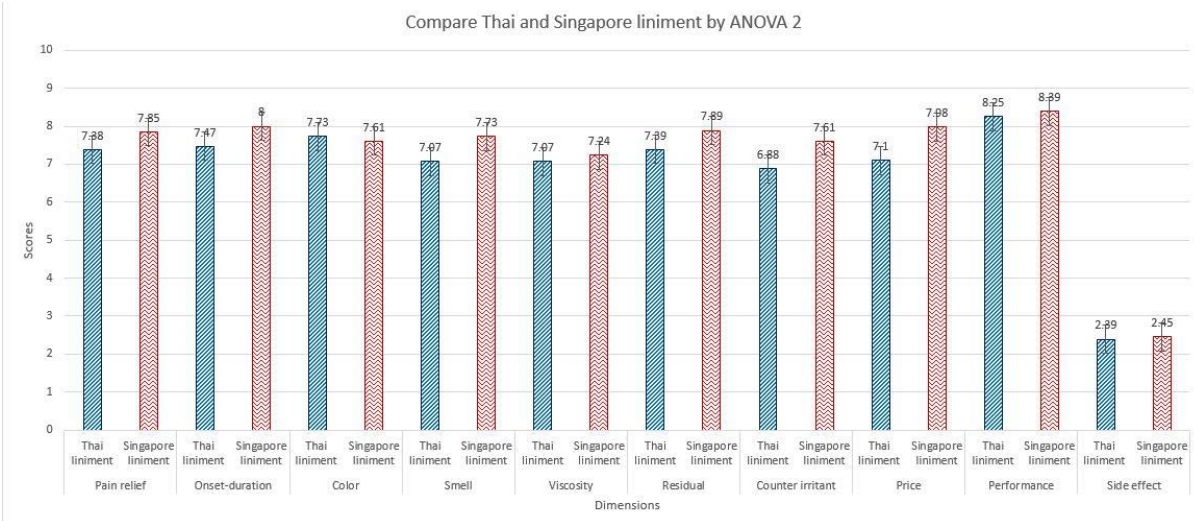

Figure 2 Compare means by the second ANOVA of Thai and Singapore liniment in 10 aspects namely; pain relief, onset and duration, color, smell, viscosity, residual, counterirritant, price, performance and side effect.

Table 2 Compare means of 10 metric measurement variables by ANOVA 2 times (crossing-over)

		First ANOVA test		Second ANOVA test	
Concepts	**Country**	**Mean±SD**	**p-value**	**Mean±SD**	**p-value**
Pain relief	Thai liniment	7.95±1.54	0.289	7.38±1.63	0.260
	Singapore liniment	7.49±1.55		7.85±1.38	
Onset-duration	Thai liniment	7.78±1.48	0.929	7.47±1.58	0.061
	Singapore liniment	7.60±1.46		8.00±1.32	
Color	Thai liniment	7.23±1.11	0.558	7.73±1.11	0.074
	Singapore liniment	7.05±1.05		7.61±1.01	
Smell	Thai liniment	7.61±1.40	0.026	7.07±1.47	0.073
	Singapore liniment	6.23±1.19		7.73±1.12	
Viscosity	Thai liniment	7.62±1.32	0.624	7.07±1.32	0.066
	Singapore liniment	7.89±1.20		7.24±1.18	
Residual	Thai liniment	7.15±1.69	0.399	7.39±1.70	0.259
	Singapore liniment	7.51±1.59		7.89±1.39	
Counterirritant	Thai liniment	7.43±1.49	0.371	6.88±1.60	0.055
	Singapore liniment	7.10±1.11		7.61±1.03	
Price	Thai liniment	7.34±1.48	*0.047	7.10±1.50	*0.042
	Singapore liniment	7.98±1.29		7.98±1.17	
Performance	Thai liniment	7.71±1.50	0.453	8.25±1.54	0.121
	Singapore liniment	8.04±1.60		8.39±1.42	
Side effect	Thai liniment	2.21±1.33	0.384	2.39±1.34	0.225
	Singapore liniment	2.15±1.01		2.45±0.91	

*$P<0.05$According to 2 Times ANOVA test by crossing-over design, only price was significantly different. Thai liniment was significantly cheaper than Singapore liniment.

Table 3 Compare means of 10 aspects of Thai and Singapore liniment in group A by Paired t-test A

	Thai liniment	Singapore liniment	
Group A	**Mean±Std. Deviation**	**Mean±Std. Deviation**	**p-value**
Pain-relief	7.49±1.55	7.85±1.38	0.170
Onset	7.60±1.46	8.00±1.32	0.680
Color	7.05±1.05	7.61±1.01	0.720
Smell	7.23±1.19	7.73±1.11	0.855
Viscosity	7.45±1.20	7.94±1.18	0.737
Residual	7.51±1.59	7.89±1.39	0.383
Counterirritant	7.10±1.11	7.61±1.03	0.822
Price	7.34±1.29	7.85±1.17	0.064
Performance	8.04±1.60	8.39±1.42	0.433
Side effect	3.88±1.00	3.33±0.91	0.058

This crossing-over experimental design confirmed by employed 2 ANOVA and 2 paired t-test that there were no significantly differences between Thai and Singapore liniment in term of pain relief, onset and duration, color, smell, viscosity, residual, counterirritant, performance and side effect ($p \geq 0.05$). However, price of Thai liniment was significantly cheaper than Singapore liniment ($p<0.05$).

Table 4 Compare means of 10 aspects of Singapore and Thai liniment in group B by Paired t-test B

	Thai liniment	**Singapore liniment**	
Group A	Mean±Std. Deviation	Mean±Std. Deviation	p-value
Pain-relief	7.95±1.54	7.38±1.63	0.147
Onset	7.56±1.48	7.19±1.58	0.602
Color	7.23±1.11	6.73±1.11	0.741
Smell	7.61±1.40	7.07±1.47	0.711
Viscosity	7.62±1.32	7.07±1.32	0.678
Residual	7.90±1.69	7.39±1.70	0.382
Counterirritant	7.43±1.49	6.88±1.60	0.780
Price	7.54±1.48	7.10±1.50	0.064
Performance	7.71±1.50	7.25±1.54	0.414
Side effect	3.16±1.33	3.59±1.34	0.058

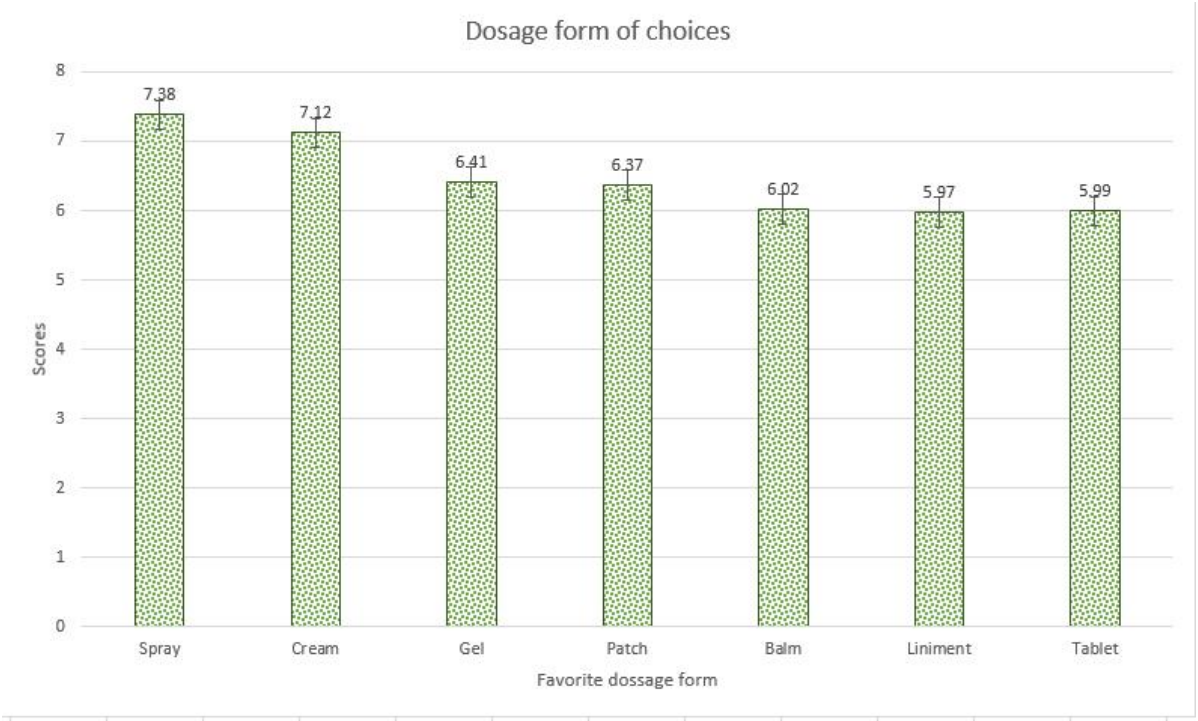

Figure 3 Dosage form of choices scores confirmed that sport-man liked spray > cream > gel > patch > balm > liniment > tablet

4. Conclusion

We found that Thai liniment brand and Singapore liniment brand liniment were not significantly different in most aspects namely, effectiveness, satisfaction and consumer risk, however Thai liniment was cheaper than Singapore liniment. Therefore, Thai government should support Thai small industry likes the OTOP liniment products to compete and survive in international market.

5. Acknowledgements

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Bioactive content, anti-tyrosinase, and antioxidant activities of ethanolic Giant Granadilla (*Passiflora quadrangularis* L.) fruit extracts for cosmetic applications

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Abstract

The *Passifloraceae* family has received attention in recent years due to its unique and excellent fruit flavor, and health benefits. Giant Granadilla (*Passiflora quadrangularis* L.) is the largest fruit in its genus (1-3 kg), therefore has gained attention from local growers. The objectives of this research were to study the bioactives, antioxidant activity and anti-tyrosinase activity of *P. quadrangularis* extracts by comparing different parts of the fruit (epicarp, mesocarp, endocarp, and seed) and develop skincare product containing *P. quadrangularis* extract. The experiment was performed by analyzing the total phenolic content, total flavonoid content, anti-tyrosinase, and antioxidant activities with DPPH and ABTS methods of the ethanolic extracts. The results revealed that the epicarp extract contained the highest amount of total phenolic content (32.57±0.02 mg GAE/g extract), whereas the seed extract contained the highest amount of total flavonoid content (113.29±0.07 mg QE/g extract). The study of antioxidant activity revealed that the seed extract had the lowest IC_{50} value for both DPPH and ABTS assays. While the study of anti-tyrosinase activity revealed that the epicarp and endocarp extract had the highest % inhibition value by using L-tyrosine (83.70±0.83%) and L-DOPA (88.43±0.50%) as substrates, followed by the seed and mesocarp, respectively. The skincare product containing *P. quadrangularis* fruit extract from the epicarp and endocarp part was formulated. The formulation exhibited acceptable physical characteristics and good stability under accelerated conditions. The cream texture was yellowish color with a pH of 5.6. Subsequently, the safety was evaluated on 20 healthy volunteers, the result showed non-irritating and good cutaneous compatibility. A satisfactory survey was implemented using a questionnaire, and volunteer satisfaction scores were high for the product's texture and moisturizing efficacy. This paper presents the fruit value and profitability in agribusiness for phytocosmetic as a multifunction ingredient.

Keywords: Antioxidant; Anti-tyrosinase; Giant Granadilla; *Passiflora quadrangularis* L; Skincare

1. Introduction

Giant granadilla fruit (*Passiflora quadrangularis* L.) belongs to the family *Passifloraceae.* The *Passiflora* fruit has received attention worldwide due to its fresh, unique aroma and flavor which are the results of natural combinations of volatile constituents in a well-balanced system including minerals, sugar, organic acids, and phenolic compounds. The giant granadilla plant is native to South America. It grows well in hot and humid climates. The optimum temperature is 18–30 °C. Fruits can be harvested from July to October (Morton, 1987). Approximately 100 g of the giant granadilla fruit contains 70.8 mg of vitamin C, 0.8 mg of iron, 17.1 mg of phosphorus, 0.378 mg of vitamin B3 and 13.8 mg of calcium, respectively (Health Benefits Times, 2020). Ramaiya *et al*, (2021) studied the antioxidant activities and secondary metabolites of the *P. quadrangularis* fruit. Findings revealed that the mesocarp and endocarp parts of the fruit have phenolic and flavonoid compounds that are associated with antioxidant activity including eriocitrin and isopropyl methoxycinnamic acid. According to the study by Guevara *et al*, (2019), giant granadilla was extracted with hydroalcoholic substances. The total phenol content, total flavonoid content, and vitamin C content were determined and found of 3.24±0.36 mg GAE/g FW, 1.79±0.24 mg Cateq/g FW, and 2.77±0.22 mg Vit C/g FW, respectively. Total antioxidant activity was then analyzed by the FRAP and DPPH methods, found of 387.89±33.11 and 90.68±8.05 µmol TEq/g FW, respectively. Matsui *et al*, (2010) investigated the activity of piceatannol, which is the phenolic compound in the *P. edulis* seed extract that has antioxidant activity by scavenging reactive oxygen species (ROS) free radicals in the body, resulting in the inhibition of melanin production and reducing the number of matrix metalloproteinases-1 (MMP-1), which is an enzyme responsible for breaking down collagen. The increase of collagen and elastin causes an increase in moisture content within the skin. Piceatannol also has an inhibitory effect on the tyrosinase enzyme, which is the main enzyme in melanogenesis, causing melanin inhibition and brightening the skin. Ingale and Hivrale, (2010) reported the pharmacological activity of different parts of passion fruit such as leaves, flowers, stems, and fruits due to the presence of important substances in the group of glycosides, alkaloids, phenols, and glycosyl flavonoids. Therefore, the giant granadilla fruit which belongs to the same family together with passion fruit has the potential to be an active ingredient in the cosmetic industry that will increase the value and the profitability of agribusiness.

From the researcher's study reports above, extracts from various parts of the fruit are exhaustively conducted for accessibility achievement towards low-cost antioxidants containing such as polyphenol, flavonoids, vitamins, and minerals improving health quality accordingly. Recovery of bioactive compounds from agri-food industry waste for pharmaceutical, food industry, and cosmetics is currently of interest. As in the parts of the passion fruit that can help add moisture to the skin. stimulate collagen production and inhibit melanin formation. However, due to a lack of data on research studies on giant granadilla extract and its use in cosmetic products. Therefore, the aims of this research were to study the bioactives, antioxidant activity, and anti-tyrosinase activity of *P. quadrangularis* extracts and develop skincare product containing *P. quadrangularis* extract.

2. Materials and Methods

2.1 Preparation of *P. quadrangularis* fruit extract

The giant granadilla fruit (*Passiflora quadrangularis* L.) was cultivated by the Chiang Rai Community Enterprise Farmers Group in Chiang Rai, the northern province of Thailand, and received in October 2022. All parts of fresh fruits were separated. Various parts such as peel (epicarp or exocarp), mesocarp, endocarp, and seed were washed and dried in a hot air oven at 45°C for 3-4 days until dry, then each part was crushed to reduce the size and stored in a sealed container. The dried plant parts were then weighed in the amount of 100-150 g and extracted by maceration method using 95%v/v ethanol as the solvent at a 1:2 ratio for 72 h at room temperature. After that, each part of the extract was filtered and evaporated the ethanol using a rotary vacuum evaporator, where different parts of the extract can be calculated for the percentage yield (% yield).

2.2 Determination of total phenolic content

The *P. quadrangularis* extracts were determined for the total phenolic content by using the Folin-Ciocalteu's reagent following the protocol of Pourmorad *et al,* (2006) with some modifications. The gallic acid standard solution was prepared with a serial dilution range of 10-60 mg/ml and the sample solution at a concentration of 1 mg/ml in absolute ethanol. Then pipet 1 ml of sample solution into a test tube and add 4 ml of 7.5%w/v sodium bicarbonate and 2 ml of Folin-Ciocalteu's reagent, mix well and stand for 30 min. The absorbance was measured at a wavelength of 765 nm by UV-Vis Spectrophotometer. The total phenolic content in each extract was compared with the standard gallic acid and expressed as milligram gallic acid equivalents per gram extract (mg GAE/g extract).

2.3 Determination of total flavonoid content

The extracts were determined for total flavonoid content using the method of Pourmorad *et al,* (2006) with some modifications. The quercetin standard solution was prepared with a serial dilution range of 100 - 500 mg/ml as well as a sample dilution of 1-10 mg/ml in absolute ethanol. Then pipet 1 ml of sample solution into a test tube, add 4 ml of distilled water and 0.3 ml of 5%w/v $NaNO_3$, then stand for 5 min. After that, add 0.3 ml of 10%w/v $AlCl_3$ and stand for 1 min. Next, add 2 ml of 1 M NaOH and 10 ml of distilled water. The absorbance was measured at a wavelength of 510nm by UV-Vis Spectrophotometer. Total flavonoid content was calculated compared with the standard quercetin and expressed as milligram equivalents of quercetin per gram extract (mg eq QE/g extract).

2.4 Assessment of antioxidant activities

2.4.1 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) radical scavenging assay

The scavenging activity of the extracts on DPPH radical was determined using the method of Pourmorad *et al,* (2006) with some modifications. The DPPH solution was prepared to contain 0.0066 g in 100 ml of absolute ethanol and sample at a concentration of 0.039 - 10 mg/ml in absolute ethanol. The 20 µl of each sample in a 96-well plate was allowed to react with 180 µl of DPPH at room temperature for 30 min in the dark. The absorbance was measured at a wavelength of 520 nm with a microplate reader and the IC_{50} was calculated compared with the Trolox standard.

2.4.2 2,2'-azino-bis(3-ethylbenzothaiazoline)-6-sulfonic acid (ABTS) assay

The ABTS scavenging activity of the extracts was determined by using the method of Pourmorad *et al,* (2006) with some modifications. The stock 7 mM ABTS solution and 140 mM potassium persulfate solution were prepared in deionized water (DI water). Then mix these two prepared solutions together and store in a light protection container for 16 h. Before use, the solution was diluted with approximately 80-120 ml of absolute ethanol to obtain an absorbance in the range of 0.5-0.8. In the assay, prepare the sample solution at a concentration of 0.1953 - 10 mg/ml in absolute ethanol, then pipette 2 µl of sample solution into a 96-well plate and add 200 µl of ABTS solution. The absorbance at 734 nm was determined after 6 min of mixing. The IC_{50} was calculated compared with the Trolox standard.

2.5 Assessment of anti-tyrosinase activity

The tyrosinase enzyme inhibition of the extracts was determined using the method of Yoshimura *et al,* (2005) with some modifications. The sample solution was prepared at a concentration of 5 mg/ml in 20%v/v DMSO. Then, 70 µl of the sample solution is mixed with 70 µl of 20 mM sodium phosphate buffer (pH 6.8) solution and 70 µl of 500 U/mL of mushroom tyrosinase in a 96-well plate. The mixture was incubated at room temperature for 10 min. After that, add 70 µl of 0.85 mM L-tyrosine or L-dopa to each well. Shake well and incubate at room temperature for 20 min. The absorbance was measured at a wavelength of 492 nm with a microplate reader and using kojic acid as a standard.

2.6 Preparation of skincare product containing *P. quadrangularis* extract

The extract from the part of *P. quadrangularis* fruit which has high bioactive content and excellent activities was selected to prepare the formulation. The skincare product was performed by preparing an oil phase and an aqueous phase. Each phase was heated over a water bath to about 70 °C, then the aqueous phase is slowly added to the oil phase while stirring continuously by using a homogenizer until homogeneous. The formulation was left cool at room temperature. The extract was mixed with the cream base in the form of an emulsion at a concentration of 1%. The cream base contains purified water, jojoba oil, 1,3-butylene glycol, glycerine, isononyl isononanoate, glycereth-26, carbopol 940, phenoxyethanol, triethanolamine, allantoin, and liquid polymer.

2.7 Stability study

The formulation was tested under accelerated conditions by heating-cooling cycling. The formulation was stored in an incubator at 45°C for 48 h, and then refrigerated at 4°C for 48 h, repeated for 6 cycles. To determine the stability under actual storage conditions, the product was stored at room temperature (30 °C/75% RH) for 1 month. The physicochemical properties including appearance, viscosity, and pH value were recorded.

2.8 Clinical study of safety and satisfaction assessment of the skincare product

2.8.1 Clinical evaluation of volunteers

The study protocol was approved by the Human Research Ethics Committee of the Faculty of Pharmacy Chiang Mai University (No. 44/2563) and in accordance with international standards (Cosmetics Europe, 2004). The evaluation was performed on 20 volunteers aged 25-55 years. All participants did not have a history of skin allergy, were not under any treatment or receiving any medical drugs, not during pregnancy or breastfeeding, and had no history of allergy to any ingredients in the formulation. Before being enrolled in the study, each volunteer received the information protocol that contained information about the products and study methods and signed an informed consent form.

2.8.2 Safety evaluation

To confirm the safety of the products, a patch test protocol was used for the evaluation. After cleaning the experimental area with deionized water, the Finn chamber® occlusive patch containing the test product, 2%v/v extract, and 2%w/v sodium lauryl sulfate (SLS) was applied to the forearm of the participants. After 4 h of application, the Finn chamber® was removed. The erythema and edema parameters were observed and recorded the relevant scores. The evaluation was obtained immediately after removing the patch and at 24 h, 48 h, and 72 h after removing the patch, then calculated the primary dermal irritation index (PII).

2.8.3 Satisfaction Assessment

After completing the skin irritation study and excluding the participants that did not compile the results, a satisfaction assessment by questionnaire was conducted to survey the satisfaction of the participants with the skincare product. The questionnaire for satisfaction assessment of the test product was developed based on product appearance, performance, and how the participants felt about the product after applying the product on the forearm once daily for 28 days. Satisfaction scores were divided into five levels and expressed as a percentage.

2.9 Statistical Analysis

Data were presented as the mean ± standard deviation (SD) and a one-way ANOVA test was used to evaluate the difference between groups using the program SPSS version 16.0. The level of statistical significance was $p < 0.05$.

3. Results and Discussion

The current passion fruit market has a tendency towards healthy and functional products at affordable prices (Das et al., 2013). The demand has increased not only because of its sweet and characteristic flavor but also due to its essential nutrient composition and antioxidant properties (Phamiwon et al., 2016; Ramaiya et al., 2019). Recently, *P. quadrangularis* L., known as the giant granadilla, has gained attention from local growers due to its relatively large fruit size (~1-3 kg), aromatic flavor, and health benefits. *Passiflora* species are rich in phytochemicals such as flavonoids, tannins, phenols, glycosides, fatty acids, and alkaloids (Ingale & Hivrale, 2010).

3.1 The *Passiflora quadrangularis L.* fruit extracts

The *P. quadrangularis* fruit and different parts of the ethanolic extracts are shown in **Figure 1**. Where the epicarp was a brown viscous extract, the mesocarp was a dark yellowish viscous extract, the endocarp was an orange-brown viscous extract, and the seed was a dark brown viscous extract. The different parts of the extract can be calculated for the percentage yield (% yield) and reported in **Table 1**. It was found that the endocarp extract has the highest percentage yield (25.22%), followed by the mesocarp (23.62%), epicarp (8.67%), and seed extract (6.00%), respectively.

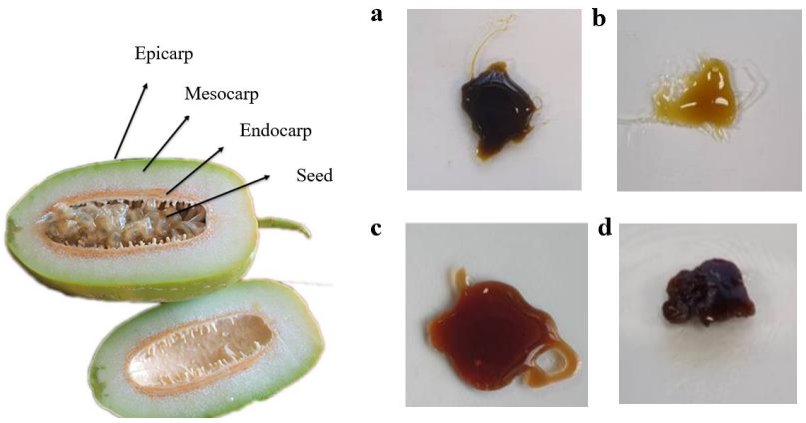

Figure 1 Giant granadilla (*Passiflora quadrangularis* L.) and different parts of the ethanolic extracts: (a) Epicarp, (b) Mesocarp, (c) Endocarp, (d) Seed

3.2 Bioactive content and biological activities of *P. quadrangularis* fruit extracts

The determination of total phenolic and total flavonoid contents is associated with the antioxidant activity of naturally derived compounds (Craft *et al*, 2012). Total phenolic content and total flavonoid contents were determined and characterized for quality control and standard aspect. The total phenolic content of the different parts of the ethanolic extracts was calculated from the gallic acid standard curve (R^2 = 0.9976), and the total flavonoid content (R^2 = 0.9908) as shown in **Table 1**. The results showed that the epicarp extract has the highest total phenolic content (32.57 ± 0.02 mg GAE/g extract), followed by the seed, endocarp, and mesocarp extract, respectively. Whereas, the total flavonoid content of the seed extract was highest far apart compared with other parts (113.29± 0.07 mg QE/g extract), followed by the endocarp, epicarp, and mesocarp extract, respectively. There were significant differences between each part of the fruit extracts.

Free radical scavenging activities in plant extracts are widely evaluated by ABTS and DPPH assays due to their similar mechanism on a single electron transferring efficiency. These assays were therefore conducted in parallel to confirm the activity (Lourith and Kanlayavattanakul, 2013). From the study of the antioxidant activity of various extracts of the *P. quadrangularis* fruit by DPPH and ABTS methods, the results were reported as IC_{50}, which means the test substance concentration that can inhibit free radicals by 50%. The low value of IC_{50} showed a high antioxidant capacity. It was found that seed extract had the best inhibitory effect on DPPH (3.65± 0.02 mg/ml), followed by epicarp, endocarp, and mesocarp extracts,

Table 1 Extraction yield and bioactive content of ethanolic *P. quadrangularis* L. fruit extracts.

Sample	%Yield	Total Phenolic content (mg GAE/g extract)	Total flavonoid content (mg QE/g extract)
Endocarp	25.22	27.47±0.00^{c}	19.44±0.01^{b}
Epicarp	8.67	32.57±0.02^{a}	14.70±0.01^{c}
Mesocarp	23.62	4.54±0.00^{d}	5.521±0.03^{d}
Seed	6.00	28.28±0.01^{b}	113.29±0.07^{a}

Values are given as mean ± S.D. from triplicate.
Different letters in the same column indicate significant differences ($p<0.05$).

respectively. As a result of the ABTS assay, the seed extract also had the best inhibitory effect (6.61±0.38 mg/ml), followed by mesocarp, epicarp, and endocarp extracts, respectively. This may be due to the higher content of free radical inhibitors such as phenolic and flavonoid compounds in the seed extract than the other parts. However, the antioxidant potency was less than Trolox, which was used as a positive control (**Table 2**).

The tyrosinase enzyme is a key enzyme for melanin synthesis that catalyzes the oxidation of the L-tyrosine to L-DOPA with subsequent transformation to melanin pigment. The tyrosinase inhibitory effect was evaluated for the application for prevention of darkening of human skin. The results showed that the epicarp extract exhibited the most tyrosinase inhibitory effect by using L-tyrosine as a substrate (83.70±0.83%), followed by the seed, endocarp, and mesocarp extract, respectively. While the results of the analysis of tyrosinase inhibition by using L-DOPA as a substrate found that the endocarp extract had the highest %inhibition values (88.43±0.50%), followed by the epicarp, seed, and mesocarp extract, respectively. It has been shown that the mechanism of tyrosinase inhibition has several sites. However, the activity of the extracts was less than kojic acid, which was used as a positive control as shown in **Table 2**.

Table 2 Antioxidant and anti-tyrosinase activities of ethanolic *P. quadrangularis* L. fruit extracts.

Sample	DPPH	ABTS	%Tyrosinase inhibition (Conc. 5 mg/ml)	
	(IC_{50}±SD, mg/ml)		L-tyrosine	L-DOPA
Endocarp	8.60±0.19^{b}	27.06±1.05^{a}	29.22±1.07^{d}	88.43±0.50^{b}
Epicarp	4.22±0.60^{c}	17.67±0.01^{b}	83.70±0.83^{b}	77.05±0.13^{c}
Mesocarp	12.35±0.17^{a}	15.60±0.43^{c}	18.65±0.33^{e}	27.66±0.47^{e}
Seed	3.65±0.02^{d}	6.61±0.38^{d}	52.36±0.75^{c}	42.32±0.17^{d}
Trolox	0.08±0.00^{e}	0.65±0.04^{e}	-	-
Kojic acid	-	-	100±0.00^{a}	96.66±0.13^{a}

Values are given as mean ± S.D. from triplicate.
Different letters in the same column indicate significant differences ($p<0.05$).

3.3 Formulation development of skincare product containing *P. quadrangularis* extract

The skincare product containing *P. quadrangularis* extract was formulated by using both epicarp and endocarp extracts because of the highest yield and bioactive content with excellent anti-tyrosinase activity. The product has a yellowish color with a pH value of 5.6. The stability test was tested under accelerated conditions by using Heating-Cooling cycling and at room temperature (30 °C/75% RH). The Heating-Cooling of a total of 6 cycles was performed as shown in **Table 3**. The product was stable without creaming or sedimentation. The color was slightly darkened after storage under accelerated storage, as shown in

Figure 2. While the pH and viscosity were slightly changed, but there were no statistically significant differences ($p > 0.05$).

Table 3 The stability study of the skincare product containing *P. quadrangularis* extract

Storage condition	pH	Viscosity (Pa.s)
Day 0	5.60±0.00	0.425±0.01
Heating-Cooling cycle (6 cycles)	5.58±0.01	0.410±0.06
30 °C/75% RH (1 month)	5.60±0.00	0.415±0.07

Values are given as mean ± S.D. from triplicate.

Figure 2 The stability of the skincare product under different storage conditions: (Left) Day 0, (Center) Heating-Cooling cycle, (Right) Room temperature

3.4 Clinical study of safety and satisfaction assessment of the *P. quadrangularis* extract cream

An irritation test on healthy volunteers was performed to guide the safety of the product application. From the patch test results, it was found that the PII index of the extract and skincare product was 0.23 and 0.15, which indicated no irritation in the volunteers compared to 2%w/w SLS (0.50), which was classified as being slightly irritated.

The satisfaction of the volunteers was evaluated after 28 days of using the skincare product as shown in **Figure 3**. The topics to evaluate satisfaction include the product appearance and perception of the skin condition of the volunteers. The satisfaction assessment was divided into five levels. Level 1 was very dissatisfied, and level 5 was very satisfied. The results of the overall satisfaction were that 45% of the subjects were very satisfied and satisfied with the product, indicating the cream is pleasant to use and acceptable to consumers. For the appearance of the product, 80% reported that they were satisfied or very satisfied with the cream texture. In the perception of the skin condition, most of the volunteers reported that they were satisfied or very satisfied with the does not stickiness, gives skin hydration, gives soft and smooth skin, and does not skin irritation.

4. Conclusion

P. quadrangularis extract contains bioactive compounds including phenolics and flavonoids. The epicarp extract had the most total phenolic content significantly higher than the other parts, whereas the seed extract had the highest total flavonoid content far apart from the other parts. For the analysis of the activities, the most potent antioxidant was the seed extract significantly better than the other parts for both DPPH and ABTS assays. Additionally, the epicarp extract exhibited the most tyrosinase inhibitory effect by using L-tyrosine as a substrate, while the endocarp extract had the most tyrosinase inhibitory effect by using L-dopa as a substrate. The skincare product containing epicarp and endocarp extracts of the *P. quadrangularis* fruit was successfully developed and exhibited acceptable physical characteristics and good stability under accelerated conditions. The product did not cause irritation in the volunteers and indicated

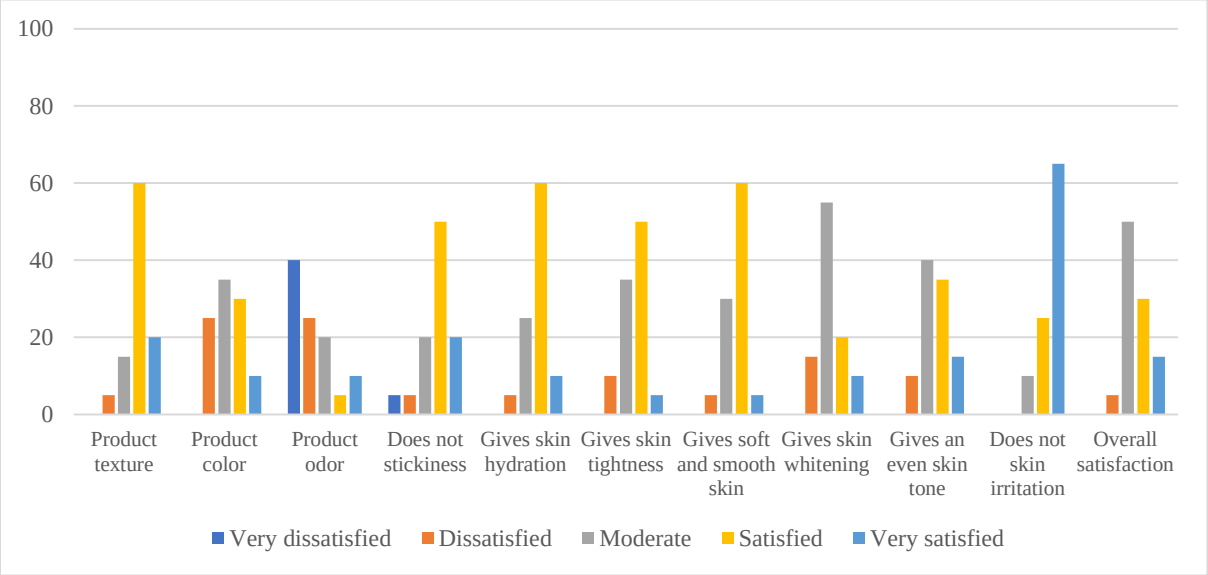

Figure 3 Percentage of satisfaction with appearance and the perception of the skin condition of the volunteers after using skincare product containing *P. quadrangularis* extract

the product was safe. The satisfaction assessment of the volunteers on the overall product was that 45% of the subjects were very satisfied and satisfied with the product, which was in the moderate satisfaction range but was in the high range for the product's texture and moisturizing efficacy. In conclusion, *P. quadrangularis* fruit is valuable and profitable in agribusiness for phytocosmetic as a multifunction ingredient.

5. Acknowledgements

This research was financially supported by the Industrial Research and Technology Capacity Development Program (IRTC No.195/2021) Northern Science Park (CMU STEP).

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Production and antioxidant activity of pectin-oligosaccharides from early immature durian fruit

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Abstract

Early immature durian (*Durio zibethinus* L.) fruit (30-45 days after anthesis with 6-12 cm in length) is considered as an agricultural waste from cultivation. It has been known that durian rinds of mature fruit contain pectin. However, the early immature durian (EID) fruit has not been reported if it contains pectin. Therefore, the aim of this study was to confirm pectin content in the EID fruit. We performed an extraction from EID fruit using distilled water at different temperatures (25, 50, and 75 ^{o}C) for 1 h to investigate the pectin content. Instrumental analyses including X-ray diffraction (XRD) and Fourier-transform infrared spectroscopy (FT-IR) suggest that EID extract contains pectin. To increased biological activities, we utilized Viscozyme®, a mixture of carbohydrases, to degrade pectin into pectin-oligosaccharides (POS). Antioxidant activity was subsequently measured in terms of free radical scavenging capacity using the 1,1-diphenyl-2-picrylhydrazy (DPPH) and 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assays. The results revealed that the Viscozyme® -treated extract can degrade pectin to pectin oligosaccharides (POS) with improved antioxidant property. Therefore, we have successfully developed the extraction method and hydrolysis process to improve the biological activity of the extract that can be further exploited.

Keywords: Early immature durian; Enzyme hydrolysis; Pectin oligosaccharides; Antioxidant activity

1. Introduction

In Thailand, durian (*Durio zibethinus*. L) as the king of fruits, is widely consumed. Many of the early immature durian fruit (EID) (30-45 days after anthesis with the length of 6 - 12 cm) were thinned to retain the fruit's qualities, including size and flavor. Thus, the farmers prefer to cut the fruit at this stage. Therefore, it is important to convert this agricultural waste into a value-added product. It has been known that whole durian fruit is inedible consisting of the outer prickly rind and inner perigones (non-edible perianth). The two parts contain pectin as a component (Begum et al., 2014). Pectin is a common component biomass byproduct (Shalini & Gupta, 2010). Pectin is a complex and colloidal heteropolysaccharide. It is composed of structurally distinct regions or domains including homogalacturonan (HG), rhamnogalacturonan (RG-I), rhamnogalacturonan (RG-II). HG, accounting for approximately 65% of pectin, is a linear polymer of α-1,4 linked galacturonic acid that is partially methyl-esterified at C-6 and O-acetylated in positions 2 and 3. (Figure 1) (Mao et al., 2019)

Galacturonic acid and its oligomers are produced by the enzymatic degradation of pectin. Pectin oligosaccharide (POS) has various applications such a signal molecules in plant defenses and plant growth development. POS is also used in the food industry, medicine, pharmacy, and cosmetic industry. Moreover, POS, as a prebiotic, has been reported that it increased the populations of beneficial bacteria

in the human digestive tract. Likewise, the composition and structural of POS varies upon the plant source and the production process (Olano-Martin et al., 2003).

The present study investigates the individual efficiency of commercial pectinase, Viscozyme L in catalyzing the liberation of pectic oligosaccharides (POS) from polygalacturonic acid. So, the aim of this study is the investigation of pectin content from EID using distilled water at different temperatures in order to enzymatically produce POS. Using XRD and FT-IR, the result suggests that EID extract contains pectin. To improve pectin biological function, we used Viscozyme to degrade pectin into pectin-oligosaccharides (POS) (Abari et al.,2021).

2. Materials and Methods

The pruned-early immature durian fruits (~30 g of each, with the length of fruit 6-12 cm, Mon Thong cultivar) used in this study were collected in March 2020, from Sangpong durian orchard in Chanthaburi province, eastern Thailand. Commercial enzyme (Viscozyme®) was used (Merck KGaA, Darmstadt, Germany).Thin-layer chromatography (TLC) was performed on Silica Gel 60 F254 (Merck). Galacturonic acid (GalA) was purchased from TCI (Chemical Express Co., Ltd) and Di-galacturonic acid (Di-GalA) was purchased from Megazyme (Bray, Ireland). All other chemicals and reagents were of analytical grade.

2.1 Extraction of pectin from early immature durian fruits (EID)

We performed an extraction from EID fruit using distilled water at different temperatures (25, 50, and 75 ^{o}C) for 1 h to investigate the pectin content. Briefly dried EID (20 mg) was added to distilled water and the solution was then centrifuged at 1400 rpm in a rotary shaker (Innova 4000 Incubator Shaker, New Brunswick Scientific, USA). After the extraction, the extract was centrifuged (5810, Eppendorf, Germany) at 4000 rpm, at room temperature for 10 min to collect the supernatant. The solution was then treated with 95% (v/v) ethanol for pectin precipitation. Finally, precipitated pectin was filtered through Whatman No.1 Filter paper (Whatman, Germany), dried in a lyophilizer, and ground into powder. Yields were calculated according to the formula (Almagro, et al., 2019):

$$\text{Yield (\%)} = \frac{\text{weight of dried recovered pectin(g)}}{\text{weight of initial powder(g)}} \text{ x } 100$$

2.3 Characterization of pectin from EID extract

We successfully extracted pectin from early immature durian fruits to obtain EID powder with a fine powder, a brownish color, and odorless. XRD and FT-IR were used to characterize the EID powder.

2.3.1 The X-ray diffraction (XRD)

XRD patterns were acquired by an X-ray diffractometer (Bruker, Yokohama, Japan). Briefly, pectins were irradiated with filtered Cu–K (α) at a voltage of 40.0 kV and a current of 40.0 mA. The scanning level was 2° at a 2θ diffraction angle which ranged from 5° to 60° (Wathoni et al., 2019)

2.3.2 Fourier Transform Infrared Spectrometer (FT-IR)

Pectin extracted from early immature durian fruits was characterized by FTIR. The powder was scanned in the range of 400–4000 cm^{-1} modified from (Abang Zaidel et al., 2017).

2.4 Enzymatic pectic oligosaccharides (POS) production from the crude pectin extract from EID

Hydrolysis of pectin obtained from early immature durian fruits (EID) with Viscozyme ®, a mixture of carbohydrates, to degrade pectin into pectin-oligosaccharides (POS). The hydrolysis was then conducted at 10% (v/v) of the diluted enzyme/pectin solution in sodium acetate buffer (0.1 M) pH 4.5. The hydrolysis was done for a period for 2 h at 45 ◦C and the enzymes were inactivated by thermal treatment at 95 ◦C for 5 min (Babbar et al., 2016) and further analyzed POS using TLC compared with standard galacturonic acid. Thin Layer Chromatography (TLC) was performed twice in n-butanol/acetic acid/water (2:1:2) as a mobile phase for visualization, the dried spots on silica gel were sprayed with orcinol/sulfuric acid reagent (8 mg orcinol in 10 mL of 70% sulfuric acid) (Hosseini-Abari et al., 2018).

2.5 Antioxidant assay

2.5.1 DPPH assay

The antioxidative effects of obtained pectic oligosaccharides were assessed at various concentrations ranging from 1.25 to 80 mg/mL. A 0.5 mL of POS was added to 2 mL of 0.2 mM methanolic solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). A stock solution of DPPH was performed by dissolving 24 mg of DPPH (SigmaAldrich, Singapore) with 100 ml methanol and the working solution was prepared by mixing 10 ml of the stock solution with 45 ml methanol. The reaction tubes were placed for 30 min at 25 °C in darkness. Afterward, the absorbance of the samples was examined at 517 nm. All samples were analyzed in triplicate and inhibitory activity (%) was calculated using the following equation modified from (Abari et al., 2021).

$$\text{DPPH radical scavenging activity (\%)} = (A_{sample} - A_{blank}) \times 100$$

2.5.1 ABTS assay

To evaluate 2, 2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activities of OP and POSs, the method of Xu, Shen, et al. (2018) was used with some modifications. Briefly, 7 mM of ABTS solution was allowed to react with 2.45 mM of potassium sulfate (1:1, v/v) in darkness for 12–16 h to generate ABTS radical cation. The solution was diluted with deionized water to obtain an absorbance of 0.700 ± 0.02 at 734 nm. After adding 0.8 mL ABTS solution to 0.2 mL sample (1–5 mg/mL), the mixture was incubated in darkness for 6 min. Absorbance of each sample was then read at 734 nm modified from (Jayesree et al., 2021). All samples were analyzed in triplicate and inhibitory activity (%) was calculated using the following equation

$$\text{ABTS radical scavenging activity (\%)} = (A_{sample} - A_{blank}) \times 100$$

3. Results and Discussion

3.1 Extraction of EID

The pectin extraction from EID are colorless, odorless and slightly viscous.When compared to the conventional extraction method of ponkan peel using water at 25^{o}C for 2h, our pectin extraction method gives greater yield (12 and upward trend when the temperature increases (Figure 1). Consistent with the previous studies that investigated effects of extraction conditions of sweet potato, such as extraction temperatures and duration, the yield of pectin increases with increasing temperature (Hamidon et al., 2017). Our extraction method of EID also gives greater amount of pectin when compared to extracted from potato pulp (Yang, et al., 2018). These differences could be possibly due to the unique sources of pectin.

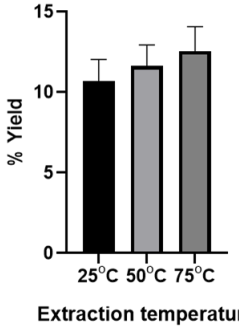

Figure 1. The EID yields at 25, 50 and 75^{o}C, respectively. The bars indicate mean ± SD of three replicates.

3.2 Characterization of pectin from EID extract

EID powder appeared as a fine powder, a brownish color and odorless. To characterization of pectin, XRD and FT IR were used.

3.2.1 X-ray diffraction study

XRD analysis is performed to investigate the crystallinity of the pectin and shows that the X-ray diffraction patterns of standard and extracted pectin are amorphous, corresponding to the previous studies. The amorphous pattern of pectin depends on intermolecular electron binding in the compounds (Wathoni et al., 2019). The results show three intense peaks at 14.28°, 24.47°, and 40.21° 2θ, indicating that our product is close to pectin (Figure 2) (Mishra et al., 2008).

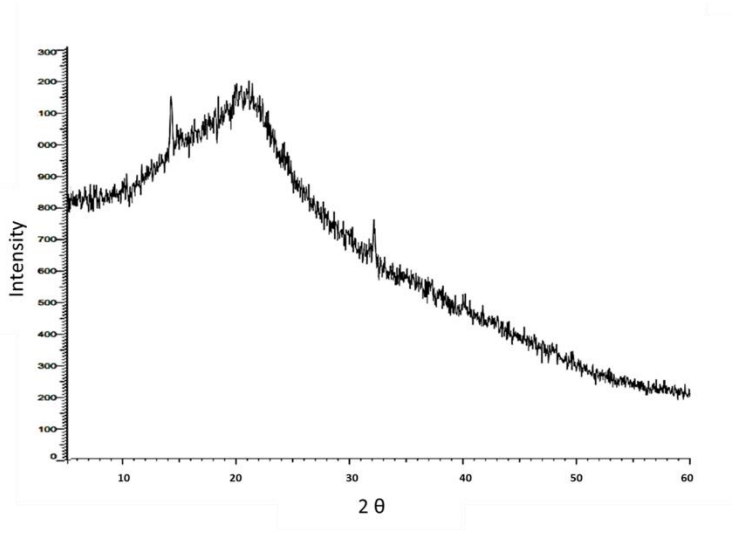

Figure 2. XRD of pectin extracted from early immature durian fruits

3.2.2 Fourier-transform infrared spectroscopy (FTIR) study

The peaks at 2947 cm^{-1} correspond to the C-H bond vibration. The peak at approximately 1700 cm^{-1} relates to the C=O bond vibration and indicates the acetyl ($COCH_3$) groups in pectin. The characteristic peak at 1609 cm^{-1} is due to the –O– tensile vibration band. The peaks at 1409 cm^{-1} represent the C–O–H in the bending vibration. In addition, there is a very weak C–O tensile vibration at 1239 cm^{-1}. The peak at 1239 cm^{-1} is asymmetric C–O–C tensile vibration and indicates the abundance of –O–CH_3 (methoxyl) groups (Figure 3). The intense peaks at 1031 cm^{-1} is the symmetric C–O–C tensile vibration (Figure 4.). We can conclude that the EID extract contains pectin as a constituent because the FT-IR spectra is similar to the previous studies, lemon peel pectin (Guolin et al., 2012).

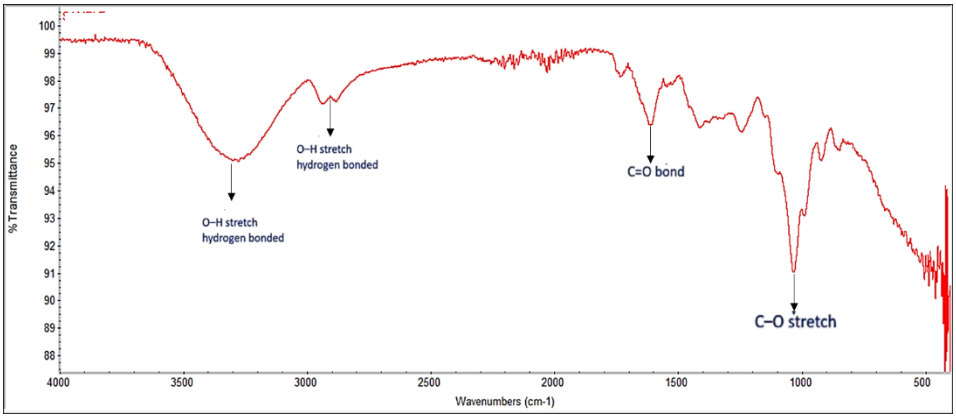

Figure 3. FT-IR spectrum of pectin from early immature durian fruits

3.3 Analysis of pectin oligosaccharide (POS) production

Thin-layer chromatography (TLC) was performed to verify the degradation of pectin. Frist spots indicate that mono, di, and tri galacturonic acid which are standards respectively. The results demonstrate Viscozyme® can successfully degrade pectin into POS. The EID extracts appeared as a dark spots mono, di and tri galacturonic acid (Figure 4).

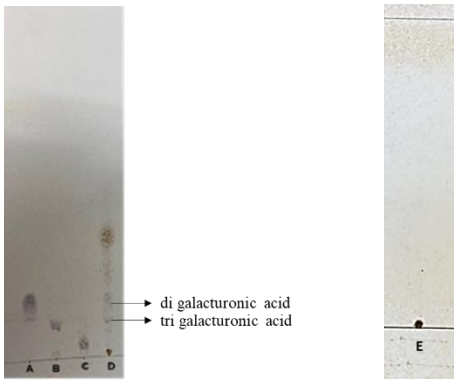

Figure 4. TLC profile of the obtained products. (A) Std. mono galacturonic acid, (B) Std. di galacturonic acid, (C) Std. tri galacturonic acid, (D) pectin oligosaccharide (POS) and (E) pectin non-hydrolyzed

3.4 Antioxidant activity of pectin and POS

Antioxidant activity of POS and pectin at the same concentrations (20 mg/mL) were tested by DPPH and ABTS assay. The POS exhibited more than 50% DPPH radical scavenging activity compared to the non-hydrolyzed pectin (Figure 5). Antioxidant activity of pectin and POS have been reported in previous studies. An antioxidant that is closely related to the presence revealed that electron-donating groups such as carboxyl, and hydroxyl can improve the antioxidant activity of phenolic compounds. The results also represented an increase in the antioxidant property of pectin after digestion by pectinase which had been confirmed in previous research (Yang et al., 2020). Although the radical scavenging activity of hydroxyl groups in polysaccharides was minor because of lacking phenolic structure, many other factors such as the presence of galacturonic acid. The chemical components in polysaccharide play an important role in the antioxidant activities of POS.

A **B**

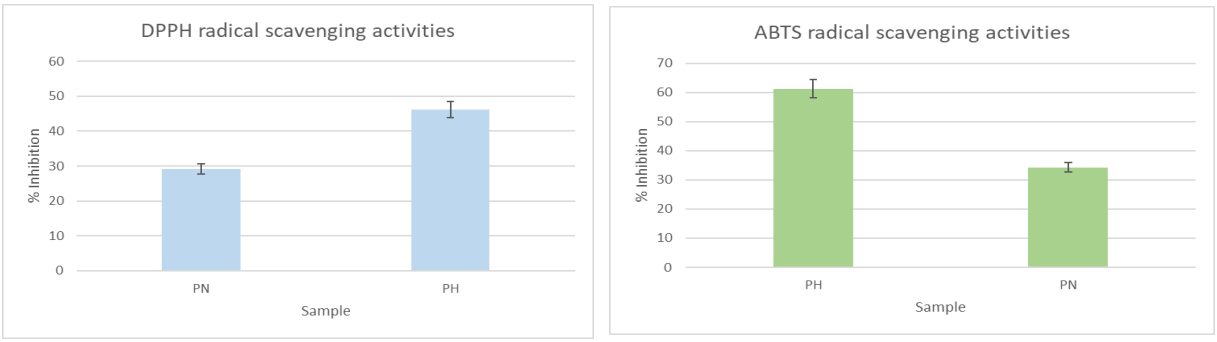

Figure 5. DPPH (A) and ABTS (B) radical scavenging activity of pectin and pectin oligo-saccharides. (PN) nonhydrolyzed pectin and (PH) hydrolyzed pectin (mean ± SD of 3replicate)

4. Conclusion

In this study, we conclude that the early immature durian fruit (EID) contains pectin as a constituent from the instrumental analysis of Fourier-transform infrared spectroscopy (FTIR) and X-ray diffraction.

Moreover, the pectin was extracted with water at different temperatures. The EID extract is harmless and suitable to be used as an active ingredient for cosmetic industries. Therefore, we have successfully developed the pectin extraction and the hydrolysis process to improve the biological activity of the extract.

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In vitro antioxidant activities and cytotoxicity of sunflower *Helianthus annuus* L. sprout extract against human skin cells

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Abstract

Sunflower (*Helianthus annuus* L.) sprouts are enriched source of caffeoylquinic acids (CQAs), including chlorogenic acid (5-CQA) and 3,5-diCQA. These compounds possess a variety of pharmacological activities, including antioxidant, anti-neurodegenerative, and anti-glycative activities. However, the biological activities being studied on human skin cells have not been reported. Here, sunflower sprout extract (SSE) was prepared using ethanol-based extraction, followed by solvent evaporation. After that, the crude extract was freeze dried to determine the antioxidant activity using chemical-based assays, as well as its cytotoxicity on human skin cell culture. SSE displayed the potent antioxidant activity by inhibiting 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2′-azinothiobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radicals with IC_{50} values of 0.332 ± 0.005 and 0.140 ± 0.002 mg/mL, respectively. To evaluate cytotoxicity of the extract, cell viability was performed by MTT assay. The results showed that SSE in all applied concentrations (0.1-1 mg/mL) had no cytotoxic effect on both immortalized human keratinocyte HaCaT and normal human BJ fibroblast cells. This study suggests that SSE exhibits a potent antioxidant with no skin toxicity, and may be considered as a pharmaceutical agent for health care products.

Keywords: Antioxidant; Caffeoylquinic acids; Fibroblast; Keratinocyte; Sunflower sprout

1. Introduction

Plants are good sources of natural products traditionally used for centuries for all personal care and cosmetic purposes (Fatima et al., 2013). Recently, bioactive extracts or phytochemicals from various plants are likely to be interesting and become more popular due to consumer concerns about synthetic ingredients or chemical substances. Therefore, it requires attention to investigate the biological properties of plant bioactive compounds (Aburjai and Natsheh, 2003).

Plant extracts are rich in phytochemicals, including phenolics, flavonoids, tannins, alkaloids, sterols, terpenoids, isoprenoids, and glycosides, with many health-promoting benefits including skin health maintenance (Mahesh, Fathima, and Veena, 2019). Among these compounds, phenolics are one of the most important groups, and caffeoylquinic acids (CQAs) comprise one of the most abundant polyphenols found in several plants, such as *Coffea* spp. (coffee; Lallemand et al., 2012), *Cynara cardunculus* L. var. scolymus (globe artichoke; Moglia et al., 2016), *Helianthus annuus* L. (sunflower; Sun et al., 2012), *Camellia sinensis* (tea) and *Ilex paraguariensis* (yerba mate; Ziemlewska et al., 2021). Further studies have shown additional benefits of CQAs, such as the reduction of cellular oxidative damage, which involve in aging-related diseases (Liang et al., 2019), neuroprotective properties from hydrogen peroxide-induced cell death (Kim et al., 2005), and inhibition of viral replication and integration (McDougall et al., 1998). According to the antioxidant role of CQAs, they showed a

protective effect against UVB-induced cellular oxidative stress in human HaCaT keratinocytes (Cha et al., 2014).

Sunflower (*Helianthus annuus* L.) sprout is abundant in phenolic compounds, trace elements, and vitamins, and thus exhibits various beneficial effects, including anti-inflammatory, antimicrobial, antioxidant, antihypertensive, and wound-healing properties (Guo et al., 2017). In a study by Cheevarungnapakul et al., (2019), Sunflower sprouts are a rich source of monoCQAs and diCQAs, particularly 5-CQA and 3,5-diCQA. Moreover, the highest antiglycation and antioxidant activities were found in the sunflower sprout extract when compared to four edible sprouts due to their rich 5-CQA and 3,5-diCQA content (Sun et al., 2012). However, a literature survey has revealed no reports on the biological activities of sunflower sprout extract obtained using ethanol-based extraction on human skin cells. Therefore, our research aimed to give more new evidence that sunflower sprout is a popular crop as a functional food and a dietary and pharmaceutical application. In the present study, we evaluated the influence of ethanolic sunflower sprout extract (SSE) on antioxidant activity and cytotoxicity against human skin cell culture. The experiments were performed on two human skin cell lines: immortalized human keratinocyte HaCaT and normal human BJ fibroblast. These cell lines typically locate in human skin layers: epidermis and dermis, respectively.

2. Materials and Methods

2.1 Sunflower sprout extract (SSE) preparation

The fresh edible sunflower (*Helianthus annuus* L.) sprouts were purchased from a local retail vegetable market in Bangkok, Thailand. The fresh samples were freeze-dried. To make the powder, samples were finely pulverized using a milling machine. Four g of freeze-dried sunflower sprout powder was extracted with 200 mL of 80% ethanol. Extract was homogenized using shaker at 250 rpm for 1 h. Supernatants were filtered using Whatman nylon membrane filter paper to remove debris and then concentrated with an evaporator at 32-37 °C. Next, the concentrated solution of sunflower sprout was dried with a freeze-dryer. The resulting crude extracts were stored at −20 °C until further use.

2.2 Determination of antioxidant activity

2.2.1 DPPH radical scavenging activity assay

Scavenging DPPH radical activity was determined by a previously described method (Herald, Gadgil and Tilley, 2012) with some modifications. First, the working DPPH solution was prepared by 10X dilution from 1.5 mM DPPH stock solution to 150 μM methanolic solution of DPPH radical. After adding 50 μl of samples to the radical solution, the mixture was then evenly combined and allowed to stand at room temperature in the dark for 30 min, then the absorbance was measured at a 517 nm. The control containing no extract was used as a base reference for the antioxidant activity. The capability of scavenging the DPPH radical was expressed as percentage inhibition using the following formula:

$$\% \text{ Inhibition} = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

IC_{50} value was defined as concentration required for inhibiting 50% radical.

2.2.2 $ABTS^{\bullet+}$-scavenging activity assay

$ABTS^{\bullet+}$ radical scavenging activity was accomplished using the standard method (Kasote *et al*., 2019) with minor modifications. The ABTS reagent was prepared by mixing equal amounts of 7.4 mM ABTS and 2.6 mM ammonium persulfate solutions and allowed to react overnight in the dark. When used in $ABTS^{\bullet+}$ working solution, the reagent was diluted to 1:40 with distilled water to achieve an absorbance of 0.7 at 730 nm. In reactions, 250 μl of $ABTS^{\bullet+}$ working solution and 50 μl of sample solution in different concentrations were mixed and incubated at room temperature for 30 min, then the absorbance was recorded in the microplate reader at 730 nm. $ABTS^{\bullet+}$-scavenging activity was calculated by a formula.

$$\% \text{ Inhibition} = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

IC_{50} value was defined as concentration required for inhibiting 50% radical.

2.3 Cell culture

Immortalized human keratinocytes (HaCaT) were purchased from ATCC. The cells were cultured in Dulbecco's modified Eagle's medium (DMEM), 2 mM L-glutamine and fetal bovine serum (FBS) were purchased from Gibco Invitrogen (Carlsbad, CA, USA) and then supplemented with 10% FBS, 100 mg/mL streptomycin and 100 U/mL penicillin. Human BJ fibroblast cells were purchased from ATCC 2522. The cells between passages 30 and 45 were cultured in minimum essential medium (MEM) with supplements as mentioned above. Both cultures are maintained in a humidified incubator with 5% CO_2 atmosphere at 37 °C, and were sub-cultured every 2 days to maintain logarithmic growth.

2.4 Cell viability assay

Cell viability was determined by 3-(4,5-dimethylthiazol)-2,5-diphenyl tetrazolium bromide (MTT; Invitrogen) assay which described by Aburjai and Natsheh, (2003). HaCaT cells (25,000-30,000 cells, 100 µL per well) and Human BJ fibroblast (8,000 cells, 100 µL per well) were seeded into 96-well microplates. Different concentrations (0, 0.1, 0.2, 0.3, 0.4, 0.8 and 1 mg/mL) of extract were added to each well of plates. After incubating plates for 24 h, the old media containing extract were removed. The addition of 5 mg/mL of MTT in phosphate buffered saline was added to wells and incubated at 37 °C for 2 h, followed by the addition of dimethyl sulfoxide to solubilize purple formazan crystals in the cells. Absorbance at 540 nm was then measured using a microplate reader to calculate percentage of cell viability from this following equation:

$$\% \text{ Cell viability} = [A_{sample} / A_{control}] \times 100$$

2.5 Statistical Analysis

Data are expressed as mean ± standard deviation (SD) of three independent experiments. The results were statistically analyzed using software GraphPad Prism v6 for Windows (GraphPad Software Inc., La Jolla, CA). All experiments were analyzed by one-way analysis of variance (ANOVA) and at $p<0.05$ level. Dunnett's test was then used to compare each experimental group with the control group.

3. Results and Discussion

3.1 Antioxidant activities of SSE

Phenolic compounds exhibit their antioxidant activity through their radical scavenging effects. Radical scavenging activity is critical due to the harmful effects of free radicals in biological systems and generally proceeds via hydrogen atom transfer or donation of electrons (Niki and Noguchi, 2000). To determine SSE's free radical scavenging activity, we used two types of radicals, DPPH and ABTS. In the present study, different concentrations of SSE were subjected to the DPPH and ABTS analyzes. As a result, the measurement of 50-percent inhibitory concentration or IC_{50} of the extract was conducted. All concentrations of extract exhibited an increasing trend of free radical scavenging ability (Figure 1). SSE displayed potent antioxidant activity by scavenging DPPH and ABTS radicals with IC_{50} values of 0.332 ± 0.005 and 0.140 ± 0.002 mg/mL, respectively. In addition, the highest capacity of SSE showed above 70% and 90% for inhibiting DPPH and ABTS radicals. This result showed different highest percent inhibition capacities of radicals since both assays differ in their response to antioxidants even though they are performed to estimate the free radical scavenging activity of a sample and based on the reduction of these radicals same (Oh et al., 2013). The antioxidant activity in sunflower sprout ethanol-based extract is influenced by CQAs, particularly 5-CQA and 3,5-diCQA. These compounds exhibited antioxidant activity (Sun et al., 2012). The previous study of *Helianthus tuberosus* L. (Jerusalem artichoke) extract by Yuan et al., (2012) showed its high CQAs accompanied by strong free radical scavenging abilities. Additionally, 5-CQA and 3,5-diCQA, found in the extracts from yerba mate showed a similar free-radical scavenging capacity and antioxidant activity to the SSE (Ziemlewska et al., 2021).

3.2 Effect of SSE on the viability of HaCaT and human BJ fibroblasts

Cytotoxicity experiments are generally designed to determine the maximal non-toxic concentration or the highest concentration compatible with cell survival of compounds, including plant extracts (Tolosa, Donato, and Gómez-Lechón, 2015). To evaluate the potential cytotoxicity of SSE, an MTT

assay was used. This assay allows the assessment of cell viability and the determination of metabolic activity from working in NAD(P)H-dependent cellular, which involves mitochondrial respiratory (Chelliah and Oh, 2022). Moreover, Laksmitawati et al., (2017) described that cell cytotoxicity was indicated by the cell viability. The cytotoxicity of SSE was evaluated on HaCaT and BJ fibroblast cell lines to identify non-cytotoxic concentrations. The non-cytotoxic concentrations of SSE showed more than 90 percent of cell viability with HaCaT and BJ fibroblast. After treatment for 24 h, the concentrations of SSE, ranging from 0.1 to 1 mg/mL, did not show cytotoxic effects on these cells when compared to the non-treated control group (Figure 2). These concentrations of SSE were considered safe for the skin, which indicated their potential use in health care products. According to dominant CQAs, 5-CQA and 3,5-diCQA, which were present in SSE including several plants, previous studies from Ziemlewska *et al*., (2021) showed that yerba mate extract had a positive effect on cell viability of HaCaT and BJ fibroblasts cells. In addition, Panichakul *et al*. (2022) reported the cytotoxic potential of *Ipomoea pes-caprae* (beach morning glory) ethanolic extract, which had both dominant CQAs. In the range of its concentrations, 0.007 to 1 mg/mL were found to have no cytotoxic effect on CCD-986sk human fibroblast cells. Based on these findings, the biological effects of SSE such as protecting against oxidative stress and enhancing skin moisturization should be further analyzed for more potential sunflower sprouts to apply on the skin.

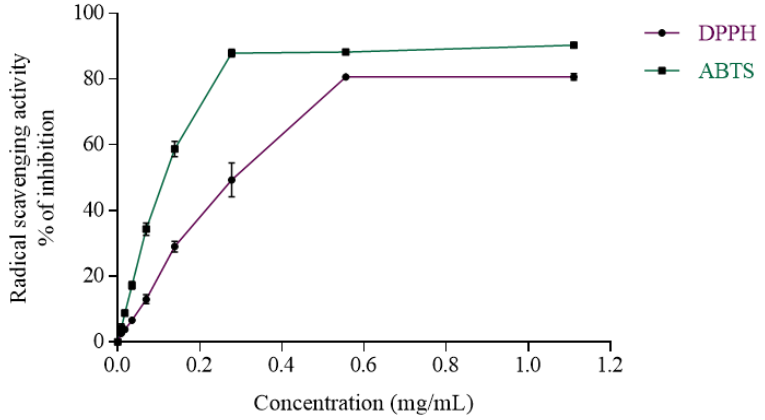

Figure 1 Antioxidant activities of extract at different concentrations was evaluated by chemical-based assays, DPPH and ABTS. Values are mean±SD of three replicate determinations (n=3).

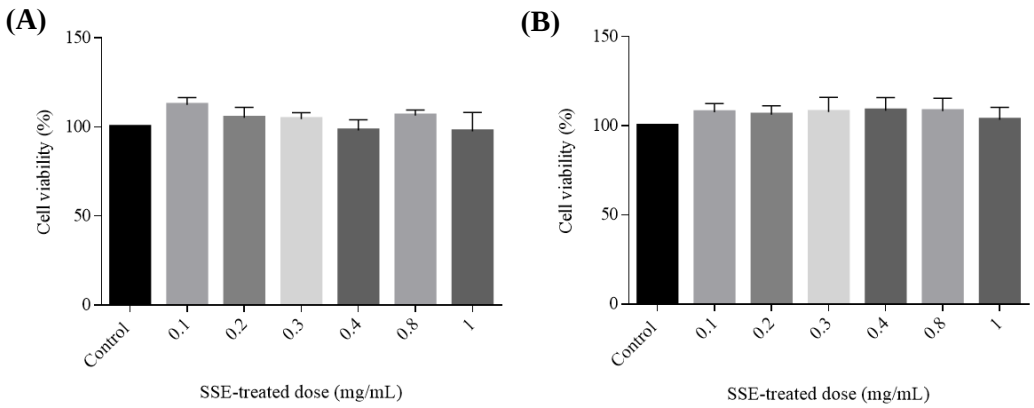

Figure 2 Cytotoxic effects of increasing in the concentrations of extract (0.1-1 mg/mL) on cell viability (MTT assay) in cultured HaCaT (A) and BJ fibroblast (B) after 24 h of exposure. Data are the mean±SD of three independent experiments, each consisting of three replicates per treatment group. $p<0.05$ versus the untreated control (100%).

4. Conclusion

This study demonstrates that in vitro bioactivity of ethanolic SSE was associated with active substances. The antioxidant properties of SSE showed that the free-radical scavenging capacity was increasing dose-dependent, which may indicate a high antioxidant potential in the extract. Moreover, further investigations showed that SSE in all applied concentrations exhibited no cytotoxic effect on skin cells: HaCaT and BJ fibroblast. These results suggest that SSE is safe for the skin and can use as a pharmaceutical agent for healthcare products. However, the limitation of this study is only based on the 2D cell culture model, therefore clinical trials are needed to ensure that the biological roles of SSE correlate with in vitro studies.

5. Acknowledgements

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Effects of Extraction Techniques on Antioxidant and Tyrosinase inhibitory Activities from *Physalis peruviana* L. fruits for using in Cosmetic Application

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Abstract

In this study, the crude ethanolic extracts of *Physalis peruviana* L. fruits from three different extraction techniques, i) Maceration technique (MPPF); ii) Soxhlet apparatus (SxPPF); iii) Sonicate apparatus (SoPPF), were evaluated for the antioxidant and tyrosinase inhibitory activities. The antioxidant capacity of each crude ethanolic extract were measured the ability by DPPH radical (2,2-diphenyl-1picrylhydrazyl) and ABTS radical (2,2'-azinobis-(3-ethylbenzothiaziline-6-sulfonate) scavenging assays. The total phenolic content was determined using the Folin-Ciocalteu method and calculated as gallic acid equivalents (GAE). The study found that the highest percentage yield (35.32 %) and total phenolic contents (GAE 1.38 mg/g of extract) were found from the SxPPF ethanolic extract. Among of the extracts from three different methods, the remarkable activity against DPPH radical (IC_{50} = 2,437.16 µg/ml) compared to ascorbic acid (IC_{50} = 165.14 µg/ml), exhibited strong $ABTS^{\bullet+}$ free radical scavenging activity (IC_{50} = 153.57 µg/ml) compared to ascorbic acid (IC_{50} = 128.92 µg/ml), and acted as potent tyrosinase inhibitor (IC_{50} = 1985.38 µg/ml) compared to the positive control kojic acid (IC_{50} = 349.36 µg/ml) were found from the SxPPF ethanolic extract. Therefore, the SxPPF ethanolic extract could be potentially used as an active ingredient in the cosmetic products according to the fast extraction method, low cost, and high potency on antioxidant activities and tyrosinase inhibitory activity.

Keywords: antioxidant activity; Physalis peruviana L.; tyrosinase inhibitor

1. Introduction

Nowadays, human beings are more conscious of health and beauty. Therefore, research studies related to the substances that promote health benefit is increasing interest, especially the research on antioxidant. The free radicals are responsible for causing a large number of diseases including cancer, autoimmune disorders, aging, cataract, rheumatoid arthritis, cardiovascular and neurodegenerative diseases (Pharm-Huy *et al*., 2008). The use of natural antioxidants is growing interest, especially in food science, complementary medicine, and cosmetics. Therefore, antioxidants are of a great benefit in improving the quality of life by preventing or postponing the onset of degenerative diseases due to oxidative stress (Mohammed *et al*., 2015).

Physalis peruviana L. (Goldenberry) is an exotic fruit that belongs to the family of Solanaceae and has been widely used as a traditional medicine since pre-Columbian times (Pigatto *et al*., 2014, Medina-Medrano *et al*., 2015), particularly in the treatment of cancer (Lan *et al*., 2009, Chang *et al*., 2008). The fruits are globose berries with smooth, waxy, orange-yellow skin and pulp that is sweet, but slightly acidic with numerous small seeds (Yildiz *et al*., 2012). The fruits have generally been regarded as food rich in vitamin C with antioxidant activities (Olivares-Tenorio *et al*., 2016). The phytochemical studies from *P. peruviana* L. fruit have indicated the presence of different chemical compounds, such as

saponins, withanolides, polyphenols, quercetin, myricetin and kaempferol which demonstrate antioxidant (Munoz *et al*., 2021, Sathyadevi *et al*., 2015).

The quantitative phytochemical analysis of ethanolic extract of *P. peruviana* L. fruit using the maceration technique contains a number of phenolic compounds (gallic acid, catechol, *p*-hydroxy benzoic acid, caffeine, vanillic acid, syringic acid, vanillin, benzoic acid, *o*-coumaric acid, *p*-coumaric acid, salicylic acid, chlorogenic acid, ferulic acid, trans-cinnamic acid, and cinnamic acid) and a number of flavonoid compounds (naringenin, kaempferol, apigenin, luteolin, naringenin and quercetin (Munoz *et al*., 2021, El-Beltagi *et al*., 2019). The quantitative analysis of the phenolic compounds reported in ethanoic extract of *Physalis peruviana* L. fruit using the High Intensity Ultrasound-assisted extraction technique identified as chlorogenic acid, caffeic acid and rutin (Coodevilla *et al*., 2018). The preliminary phytochemical analysis revealed the presence of the alkaloids, flavonoids, and phenols from the ethanolic extract of *Physalis peruviana* L. fruits using Soxhlet apparatus technique (Bharthi *et al*., 2016).

The extraction is the first step for obtaining bioactive compounds from plant materials, and conventional solvent extractions, such as maceration, percolation, Soxhlet, and stirring methods, are the most widely used. The bioactive compounds present in the fruit of goldenberry, especially phenolic compounds, have important pharmacological properties, such as antioxidant, antibacterial, and antitumor (Codevilla *et al., 2018*).

Therefore, the aims of this study were to evaluate the antioxidant and tyrosinase inhibitory capacity of the ethanolic extract of *Physalis peruviana* L. fruit extracted from three different methods: i) Maceration technique, ii) Soxhlet apparatus and iii) Sonicate apparatus.

2. Materials and Methods

2.1 Materials

The plant of *Physalis peruviana* L. fruits (PPF) of the family Solanaceae was purchased from The Royal Project, Chiang Rai, Thailand. The fruits were cleaned, cut into small pieces, and dried under shade. The dried PPF was kept in under 4^{o}C until use.

Ascorbic acid, gallic acid, kojic acid, Folin-Ciocaltues reagent, 2,2-diphenyl-l-picrylhydrazul (DPPH), and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) were purchased from Sigma-Aldrich, USA. The 125 U mL^{-1} of mushroom tyrosinase was purchased form Sigma-Aldrich, USA. The UV-Vis Spectrophotometer (Biochrom Libra S80, UK) is used for scanning the absorbance to detect the unique wavelength maxima of pigment molecules.

2.2 Extraction conditions

The extraction was performed using three different methods i) Maceration technique, ii) Soxhlet apparatus, and iii) Sonicate apparatus.

i) Maceration technique One hundred grams of sample was soaked with 95% ethanol (ratio between solid: solvent 1:2) at room temperature for 24 hours. The extract was filtered with filter paper (Whatman No. 1) while the residue was further extracted under the same condition twice. The filtrates collected form three separate extractions were combined and evaporated to dryness under vacuum using rotary evaporator (Wu *et al*., 2005).

ii) Soxhlet apparatus technique One hundred grams of sample was extracted using 95% ethanol as a solvent (ratio between solid: solvent 1:2) for 6 hours using the Soxhlet apparatus. The extract was filtered through quantitative filter paper, evaporated to dryness under vacuum using rotary evaporator, and stored under 4^{o}C until analysis (Bharthi *et al*., 2016).

iii) Sonicate apparatus technique One hundred grams of sample was extracted using 95% ethanol as a solvent (ratio between solid: solvent 1:2) for 30 minutes using Sonicate apparatus. The extract was filtered through quantitative filter paper, evaporated to dryness under vacuum using rotary evaporator, and stored under 4^{o}C until analysis (Codevilla *et al*., 2018).

The yield of the three extraction methods for *Physalis peruviana* L. fruit; i) Maceration technique (MPPF); ii) Soxhlet apparatus (SxPPF); iii) Sonicate apparatus (SoPPF), was calculated using the

formula described by Falleh (2008) as the following equation.

$$Y\ (\%) = 100\ (M_{ext}/M_{dps})$$

Where Y is the yield of extraction in %, M_{ext} is the mass of the extract (in mg) after the evaporation (using the rotary evaporator), and M_{dps} is the mass of the dried plant sample in mg.

2.3 Determination of Total Polyphenols

Total polyphenols were determined by the Folin-Ciocalteu (FC) method following Narvaez-Cuenca *et al.* 2014 method with some modification. A 100 μL extract was mixed with 125 μL of 10% (v/v) Folin-Ciocalteu's reagent. After 5 min, 25 μL of a 7% (w/v) Na_2CO_3 solution were added. After 30 min, the absorbance of the reaction mixture was measured at 725 nm. The results were expressed as gallic acid equivalents (mg GAE per 100 g of dry weight (DW) of fruit). Calibration curves for gallic acid were performed in concentrations ranging from 50-200 μg/mL ($y = 0.0820x + 0.0245$, $R^2 = 0.9983$).

2.4 Free Radical Scavenging Activities

The DPPH and ABTS Assays. The DPPH and the ABTS assays were performed according to a modified method of Narvaez-Cuenca et al. 2014. *The DPPH assay*, the ethanolic stock solution of 50 mM DPPH was prepared to obtain a working DPPH solution with absorbance intensity of 1.10 at 515 nm. The 25 μL of extract was mixed with 975 μL of working DPPH solution. The mixture was shaken vigorously and left standing at room temperature for 30 minutes in the dark, and then the absorbance was measured at 515 nm. *The ABTS assay*, the mixture of 7.0 mM ABTS solution and 2.45 mM potassium persulphate (ratio 2:1.5) was prepared and stored in a dark condition for 12-16 hours at room temperature in the dark until a stable oxidative state. The resulting $ABTS^{\bullet+}$ solution was diluted with ethanol to reach an absorbance of 0.7 at 734 nm. The reaction mixture, 1000 μL of $ABTS^{\bullet+}$ solution with 100 μL of sample solution was placed, and after agitation, it was left to rest in a dark for 15 minutes. Then, the absorbance was measured at 734 nm to assess the percentage of inhibition. The scavenging effect (in percentage) was calculated as the following equation.

$$\text{The scavenging activity} = [1-(A_1-A_0)]*100,$$

Where A_0 was the absorbance of the control (without extract), A_1 was the absorbance of the sample in the presence of the extract once the steady state plateau was reached. The amount of antioxidant activity is characterized by the value of IC_{50} which is concentration of extract required to inhibit 50% of DPPH and ABTS radicals. All samples were analyzed in three replications. In the DPPH and ABTS assays, standard curves were generated with ascorbic acid in concentrations ranging from 5 – 200 μg/mL (five data points $R^2 = 0.9959$, $y = 0.4229x + 8.4479$) and 50 – 400 μg/mL (five data points, $R^2 = 0.9919$, $y = 0.2858x - 13.156$), respectively.

2.5 Tyrosinase Inhibition Activity

The tyrosinase inhibition activity, using L-Dopa as the substrate, was performed according to the protocol described by Dej-adisai (2016) with some modifications. The red colour of dopachrome from the oxidation of L-Dopa can be detected by visible light at 492 nm. The anti-tyrosinase activity was performed in 96 well plate. The samples were dissolved with ethanol to the concentration range between 100 - 40 mg/mL. The first 20 μL of the sample's solution were mixed with 140 μL phosphate buffer (pH 6.8) and 20 μL of mushroom tyrosinase solution (203.3 unit/mL) and incubated at 25°C for 10 minutes. Then 20 μL of L-Dopa (0.85 mM) was added. The mixture solution was incubated at 25°C for 20 minutes. After incubation, the amount of dopachrome in the reaction was measured at 492 nm. The tyrosinase inhibition (in percentage) was calculated as the following equation.

$$\text{The Tyrosinase inhibition activity } [(A-B)-(C-D)/(A-B)]*100$$

Where A is the difference of optical density before and after incubation of control, B is the difference of optical density before and after incubation of blank control, C is the difference of optical density before and after incubation of test sample, and D is the difference of optical density before and after incubation of blank sample. All samples were analyzed in three replications. The absorbance of the microplate wells was read using a microplate spectrophotometer at 510 nm. The standard curves were generated with kojic acid in concentrations ranging from 150 – 500 μg/mL (six data points $R^2 = 0.9956$,

y = 0.1399x + 0.1016).

2.6 Statistical analyses

All experimental measurements were carried out in triplicate and are expressed as average of three analyses ± Standard Deviation. The differences between means were ANOVA (analysis of variance). IC_{50} value of each sample was calculated.

3. Results and Discussion

3.1 The Extract Yield and Total Phenolic Contents

As a result, the percentage yield of the ethanolic extract of *Physalis peruviana* L. from three different methods i) Maceration technique, ii) Soxhlet apparatus, and iii) Sonicate apparatus) have been shown in the Table 1. The highest extraction yield was registered in SxPPF (35.32%), followed by the extracts of MPPF and SoPPF was 26.15% and 17.99%, respectively. The total phenolic content of these extracts from *P. peruviana* L. were determined using the Folin-Ciocalteu assay by consideration a standard curve with gallic acid (GA) taking into consideration the relationship between absorbance and concentration.

Using the equation obtained from calibration curve, the SxPPF showed the highest phenolic content (1.38 ± 0.022 mg GAE/g of extract) followed by the MPPF (1.23 ± 0.025 mg GAE/g of extract) and the SoPPF (0.98 ± 0.011 mg GAE/g of extract) showed the lowest content (Table 1). The result suggests that the Soxhlet apparatus is the best technique used for the extraction of the phenolic compounds from *Physalis peruviana* L. fruit. Using ANOVA, the F value of 0.92522 and F_{crit} of 3.4668. The F cit value > F value, so it can conclude that there were no significant differences toward total phenolic content of extracts each method. From this study, it may suggest that the total phenolic content contains in all extracts may consist of polyphenols, flavonoids, and alkaloids compounds (Munoz *et al*., 2021, El-Beltagi *et al*., 2019, Coodevilla *et al*., 2018, Bharthi *et al*., 2016).

Table 1 The extract yield and total phenolic contents from *Physalis peruviana* L. fruits.

Samples	% Yield	Total phenolic contents (mg GAE/g of extract)
MPPF*	26.15	1.23 ± 0.025
SoPPF**	17.99	0.98 ± 0.011
SxPPF***	35.32	1.38 ± 0.022

*MPPF = Maceration extraction technique of *Physalis peruviana* L fruits
**SoPPF = Sonication extraction technique of *Physalis peruviana* L. fruits
***SxPPF = Soxhlet apparatus extraction technique of *Physalis peruviana* L. fruits
Values are given as mean ± S.D. from triplicate.

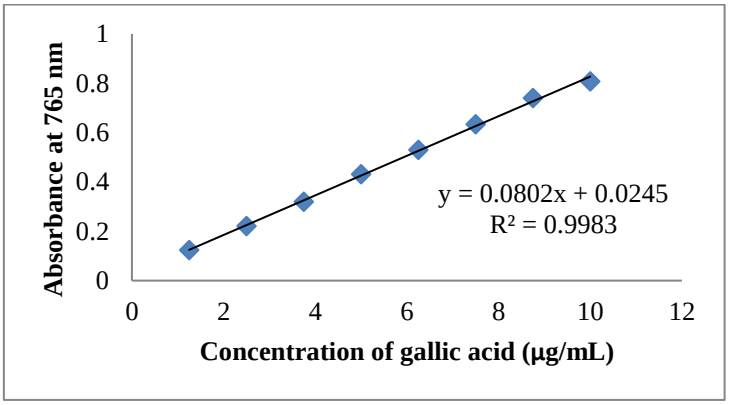

Figure 1 Gallic acid standard calibration curve for the quantification of total phenolic content.

3.2 Antioxidant Capacity

The antioxidant capacity of *Physalis peruviana* L. fruit extracted from three different extraction techniques (MPPF, SoPPF, and SxPPF) was determined by two methods, using the free radicals DPPH* (2,2-Diphenyl-l-picrylhydrazul) and $ABTS^{\bullet+}$ (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)). The results were based on the inhibition concentration at 50% (IC_{50}), compared with standard antioxidant (ascorbic acid). The calculated IC_{50} value on DPPH and ABTS free radical scavenging activities of all extracts has been shown in the Table 2. The IC_{50} value of ascorbic acid (standard) on DPPH and ABTS radical scavenging capacity was 165.14 μg/mL and 128.92 μg/mL, respectively. Among the extracts, the IC_{50} value of different extraction techniques was in the following order: SxPPF > MPPF > SoPPF (for DPPH assay) and SxPPF > SoPPF > MPPF (for ABTS assay). From the results, the *Physalis peruviana* L. fruit extract from Soxhlet apparatus technique (SxPPF) showed stronger ABTS scavenging (IC_{50} 153.57 μg/mL) than DPPH scavenging (IC_{50} 2437.16 μg/mL).

Using ANOVA, the F values of DPPH and ABTS are 0.0252 and 0.3612, respectively. The F_{crit} of both DPPH and ABTS is 3.5545. The F_{crit} value > F value, so the results indicated that there were no significant differential effects on both DPPH and ABTS free radical scavenging of extracts from each method.

Table 2 The antioxidant activity of *Physalis peruviana* L. fruits extracts from different methods and standard by DPPH and ABTS assays [IC_{50} (μg/mL).

Samples	DPPH IC_{50} (μg/mL)	ABTS IC_{50} (μg/mL)
MPPF*	2540.8	329.4
SoPPF**	2677.85	296.08
SxPPF***	2437.16	153.57
Ascorbic acid	165.14	128.92

*MPPF = Maceration extraction technique of *Physalis peruviana* L. fruit
**SoPPF = Sonication extraction technique of *Physalis peruviana* L. fruit
***SxPPF = Soxhlet apparatus extraction technique of *Physalis peruviana* L. fruit
IC_{50} = Inhibitory activity at 50%, Values are given as mean ± S.D. from triplicate.

The 2,2-Diphenyl-l-picrylhydrazul (DPPH*) assay is commonly used for determination of free radical scavenging activity of antioxidant based on the capture of the DPPH radical by the oxidants (Guine *et al*., 2020). The reveal reported by Liang and Kitts (2014) indicated that the antioxidants react with DPPH* radicals based on both hydrogen atom transfer (HAT) or single electron transfer (SET) reactions. The 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) radical cation-based assays are among the most abundant antioxidant capacity assays. The antioxidant such as phenolic nature, can form coupling adducts with $ABTS^{\bullet+}$, whereas others can undergo oxidation without coupling (Ilyasov *et al.*, 2019). The antioxidant provides an electron to the free radical ($ABTS^{\bullet+}$) and itself then become a radical cation via the SET mechanism (Liang and Kitts, 2014). In this study, the result of the free radical scavenging activity of *Physalis peruviana* L. fruit extracted from three different techniques may possess based on both HAT and SET reactions.

3.3 Tyrosinase inhibitory Activity

In this study, tyrosinase inhibitory effects of *Physalis peruviana* L. fruit on diphenolase activity of mushroom tyrosinase were evaluated. As with increasing the concentration of all extracts (All total extracts of plants inhibited tyrosinase weaker than kojic acid (IC_{50} = 356.67 μg/mL). IC_{50} values of ethanolic extract of *Physalis peruviana* L. fruit extracted from three different techniques (MPPF, SoPPF, and SxPPF) were calculated 8139.7, 8169.91, 1985.38 μg/mL, respectively (Table 3). Using ANOVA, the F values of tyrosinase inhibitory effect is 0.1145 and the F_{crit} is 4.2564. The F_{crit} value > F value, so the result indicated that there were no significant differential effects on tyrosinase inhibitory effect of extracts from each method. Among of the extracts, SxPPF showed the most tyrosinase inhibitory effects, indicating its potent tyrosinase inhibitors or high content of active compounds with tyrosinase inhibitory effects.

As reported by Chang (2009), the standard tyrosinase inhibitors classified into five major classes, including polyphenols, benzaldehyde and benzoate derivatives, long-chain lipids and steroids based on either the chemical structures or the inhibitory mechanism. Polyphenols and flavonoids isolated from plants which were identified as tyrosinase inhibitors such as kojic acid, caffeic acid, *p*-coumaric acid, kaempferol, anthocyanins, luteolin, quercetin and apigenin (Namjoyan *et al*., 2015). In this study, the tyrosinase inhibitory effects of *Physalis peruviana* L. fruit on diphenolase activity of mushroom tyrosinase may results from the total phenolic contents contains in the extracts (Munoz *et al*., 2021, El-Beltagi *et al*., 2019, Coodevilla *et al*., 2018, Bharthi *et al*., 2016).

Table 3 The tyrosinase inhibitory activity of *Physalis peruviana* L. fruit extracts from different methods and standard by Dopachrome assay [IC_{50} (μg/mL).

Samples	Total phenolic contents (mg GAE/g of extract)	L-DOPA IC_{50} (μg/mL)
MPPF*	1.23 ± 0.025	8139.7
SoPPF**	0.98 ± 0.011	8169.91
SxPPF***	1.38 ± 0.022	1985.38
Kojic acid		356.67

*MPPF = Maceration extraction technique of *Physalis peruviana* L. fruit
**SoPPF = Sonication extraction technique of *Physalis peruviana* L. fruit
***SxPPF = Soxhlet apparatus extraction technique of *Physalis peruviana* L. fruit
Values are given as mean ± S.D. from triplicate.

4. Conclusion

According to the experiment, the Soxhlet apparatus is the best technique to obtain the highest percentage while the lowest is from the sonicate apparatus. The optimum condition for the Soxhlet apparatus technique assisted extraction of *Physalis peruviana* L. fruit is 6 hours for extraction time, solid to solvent ratio is 1:2 with the maximum yield percentage of 35.32%. The crude ethanolic extract from the Soxhlet apparatus technique shows the highest total phenolic content and best antioxidant activities on the DPPH and ABTS assays, including anti-tyrosinase activity.

Therefore, the SxPPF ethanolic extract could be potentially used as an active ingredient in the cosmetic products according to the fast extraction method, low cost, and high potency on antioxidant activities and tyrosinase inhibitory activity.

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Effects of surfactants on the physicochemical properties of asiatic acid-loaded solid lipid microparticles

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Abstract

The objective of this study was to evaluate the effects of three surfactants, namely tween 80, soybean lecithin, and poloxamer 188 on the characteristics of solid lipid microparticles containing asiatic acid (AASLM). The AASLM were prepared from or 15% of beeswax together with 3% of surfactant (formulas B1-B6) by melt dispersion cum freeze-drying technique to obtain a solid dosage form. The physicochemical characteristics of the AASLM powders, including appearance, surface morphology, particle size, %labelled amount of asiatic acid (AA) and %entrapment efficiency (EE) were evaluated. The results revealed that AASLM prepared from or 15% beeswax with 3% soybean lecithin (B2 and B5) could not yield a dry powder form, whereas those with tween 80 (B1 and B4) or poloxamer 188 (B3 and B6) appeared as white to slightly yellowish coarse powders with mean particle sizes ranging from 14.15±0.11 to 38.86±0.34 microns. The surface morphology of AASLM powders, examined by scanning electron microscope, showed a non-spherical shape. The morphology of AASLM dispersions before freeze-drying and after redispersion of the freeze-dried powder were also investigated using the optical microscope. In all the dispersions, the AASLM prepared from 10% or 15% beeswax with 3% poloxamer 188 (B3 and B6) had spherical particle shape, whereas those prepared from 10% or 15% beeswax with 3% tween 80 (B1 and B4) seemed to have irregular particle shape. The %labelled amount of AA in B1, B3, B4 and B6 were 94.23±0.04, 90.43±0.02, 95.26±0.02 and 90.69±0.02, respectively, which were considered acceptable. The %EE of B1, B3, B4 and B6 were 53.75±0.01, 100.00±0.00, 51.56±0.03 and 100.00±0.00, respectively. It could be concluded that the AASLM prepared with poloxamer 188 has the greatest potential among the test formulations as a topical carrier for AA. Further studies will be conducted to explore its utilization in cosmetics.

Keywords: Asiatic acid, Solid lipid microparticles, Tween 80, Soybean lecithin, Poloxamer 188

1. Introduction

Asiatic acid (AA) is an aglycon triterpenoids or pentacyclic triterpenoid found in *Centella asiatica* which is a poorly water-soluble substance. Its water solubility is approximately 10 µg/mL (Borhan *et al*., 2013; Chen *et al*., 2020; Lv *et al*., 2018; Puttarak & Panichayupakaranant, 2012; Rafat *et al*., 2008). AA has many beneficial effects such as wound healing (Thakor *et al*., 2017; Yun *et al*., 2008), anti-inflammation, anti-oxidation (Huang *et al*., 2011; Lv *et al*., 2017; Yun *et al*., 2008) and anti-microbial activity (Djoukeng *et al*., 2005; Liu *et al*., 2015; Pojanaukij & Kajorncheappunngam, 2010), hence AA is one of the promising active ingredients used in cosmetic and pharmaceutical products.

Solid lipid nanoparticles (SLNs) and solid lipid microparticles (SLMs) are drug delivery systems which demonstrated many benefits comparing to other drug carriers. They can deliver and encapsulate active substance by solid lipid which are safe, biocompatible and biodegradable, and they can control a release of active substance to target site. It was reported that SLN loaded with AA can successfully transfer the drug (AA) to nasal route for Alzheimer's prevention and treatment. The obtaining formulas showed good physicochemical characteristics including appearance, pH, particle size, size distribution and drug loading. They were stable with no sign of precipitation under different storage conditions (4 ^{o}C, room temperature and 45 ^{o}C) for 6 months (Khunathum, 2011). Solid lipid microparticles (SLMs) are similar to SLNs in term of a composition. However, the size of SLMs (1-1000 µm) is bigger than that of SLNs (50-500 nm) (Jaspart *et al*., 2005; Pardeike *et al*., 2009; Sznitowska *et al*., 2017) which are safe for topical application. The incorporation of AA in SLMs has not been reported to date, thus SLMs can be a newly developed carrier systems for the delivery of AA for dermal application. SLMs are lipid-based systems composed of a hydrophobic core made from solid lipid. SLMs have satisfactory loading capacity of lipophilic compounds and a good affinity for the stratum corneum (Long *et al*., 2006). They also exhibited several advantages including controlled release, biocompatibility, biodegradability, low toxicity, avoidance of organic solvents in production, the possibility of large-scale production and protection of active substances from the surrounding environment (Chen *et al*., 2020; Jaspart *et al*., 2005). SLMs are potentially useful for the local delivery.

Many research studies have shown that the type of lipids, surfactants, and additives used can affect the physicochemical characteristics of the resultant SLMs (Kheradmandnia *et al*., 2010; Pietkiewicz & Sznitowska, 2004; Sznitowska *et al*., 2017; Wolska & Sznitowska, 2013). Beeswax is one of the lipids commonly used in SLM preparation. It consists mainly of esters of long chain fatty acids and alcohols (C24-C36) and small quantities of hydrocarbons, acids and other substances (Bogdanov, 2004; Tulloch, 1980; Tulloch & Hoffman, 1972). SLM made of beeswax was successfully developed as topical carriers for octyl methoxy cinnamate (OMC) (Yener *et al*., 2003), vitamin E (Souza *et al*., 2020). There are several types of surfactants used as stabilizers in SLM preparation, such as polysorbate 80 (tween 80), poloxamer 188 and lecithin (Jaspart *et al*., 2005). In 2017, Sznitowska et al. suggested that the type of surfactants used had an effect on the particle size and viscosity of the resultant SLM dispersions (Sznitowska *et al*., 2017). Therefore, the aim of this study is to examine the effects of different types of surfactants (tween 80, poloxamer 188, soybean lecithin) on the physicochemical characteristics of asiatic acid loaded in SLMs (AASLMs) prepared from beeswax.

2. Materials and Methods

2.1 Materials

Asiatic acid (95%) was obtained from SEPPIC, France. Beeswax was obtained from Kahl, (Germany), tween 80 (polysorbate 80) was purchased from Panreac applichem (Darmstadt, Germany), soybean lecithin from Merck (Germany), poloxamer 188 from BASF (Germany). All other chemicals used were of an analytical reagent grade.

2.2 Preparation of AA-SLMs

Briefly, AA was dissolved in ethanol, in ratio 0.01 g: 1.5 ml, and the solution was then added to the melted lipid phase (the melting temperature depending on the lipid used). The hot lipid mixture was then emulsified into an aqueous surfactant solution that was heated above the lipid melting point to produce the O/W emulsion. The emulsion, which is obtained by mixing with a high shear device (Ultra-Turrax® [IKA]) at 8000 rpm for 5 min, is finally allowed to cool in an ice bath (Wolska & Sznitowska, 2013). Then, the AASLMs is placed into a freeze-dryer (Labconco Lyophilizer, USA) (Zhang *et al*., 2008) to make a dry powder. The formulations of asiatic acid loaded SLMs are shown in Table 1. Freeze-drying was equipped with the condenser operating at -50 °C and a chamber with cooled shelves. The freeze-drying was conducted at a pressure of 5.0 Pa and the process lasted for 24 hours to allow a complete solidification. Dextrose anhydrous (5% w/w) was used as the cryoprotectant for the freeze-

drying process of AASLMs (Zhang *et al*., 2008).

2.3 Morphological analysis

The shapes of AASLMs before freeze-drying and after re-dispersion were inspected by using the optical microscope (Nikon Coolpix 5400, Nikon Corporation, Japan). The surface morphology of AASLMs was examined by scanning electron microscope (SEM). The samples were prepared by using cotton swabs to scoop and distribute the AASLMs powder onto the specimen stub fixed with double-sided tape. A rubber ball was used to blow away dust or non-stick particles. The specimen was then photographed under the SEM (JEOL JSM-6610LV, Tokyo, Japan) (Rahimpour *et al*., 2016).

2.4 Particle size analysis

The particle size was measured by static automated imaging technique (Morphologi 4, Malvern Panalytical, Germany). The AASLMs powder was put in a dispersed chamber and then placed inside the instrument operated at a pressure of 4 bar to disperse a sample on a quartz glass slide. All measurements were done in triplicate and data were expressed as means ± SD. The size was expressed by the circle equivalent (CE) diameter.

2.5 Entrapment efficiency and active loading

Briefly, 8 ml of the AASLM dispersion obtained at the end of the preparation method (before freeze-drying) was ultracentrifuged (Hitachi CP100NX ultracentrifugation, Japan) at 65,000 rpm for 1.5 hours at 4 °C. Then, 1 ml of supernatant (free active) was collected and filtered through 0.45 µm syringe filter and assayed by HPLC at 210 nm. In addition, the sediment was extracted by using methanol and adjusted the final volume to 5 ml. 1 ml of obtaining mixture was then filtered through 0.45 µm syringe filter and subjected to HPLC analysis. The percentage of entrapment efficiency and active loading were calculated by the following equations:

$$\% \text{ Entrapment efficiency (EE)} = \frac{(\text{W total active} - \text{W free active})}{\text{W total active}} \times 100 \quad (1)$$

$$\% \text{ Active loading (Al)} = \frac{(\text{W total active} - \text{W free active})}{\text{W lipid}} \times 100 \quad (2)$$

where W total active is total amount of AA added to the system, W free active is the analyzed amount of free active and W lipid is the weight of the lipid phase. The values were averaged on three determinations (Rahimpour *et al*., 2016).

%Labeled amount of AA in SLMs was calculated by equations below:

$$\%\text{Labeled amount} = \frac{(\text{Amount of AA in the sediment} + \text{Amount of free AA in the supernatant})}{(\text{Total amount of AA used})} \times 100 \quad (3)$$

Note: acceptable standard range of %labeled amount is 85-115% for topical drugs (USP27,2004: 2396).

2.6 Statistical analysis

All experiments were performed in triplicates (n = 3) for validity of statistical analysis. Results were expressed as mean ± SD. The statistical variance was calculated by One-way ANOVA, comparison of each group by Tukey HSD test and the differences were considered significant for p value ≤ 0.05.

3. Results and Discussion

3.1 Preparation of AASLMs powder

AASLMs were fabricated by the melt dispersion technique (Chalella Mazzocato *et al*., 2019; Sznitowska *et al*., 2017; Wolska & Sznitowska, 2013; Yadav *et al*., 2009; Zhang *et al*., 2008) in conjunction with freeze-drying, in order to obtain water-free solid particles. Beeswax, at concentrations of 10 and 15%, was chosen as lipid component and three different surfactants, namely tween 80, soybean lecithin, and poloxamer 188 were used as stabilizers. According to the results shown in Table 1 and Figure 1, the AASLM prepared from 10% or 15% beeswax with 3% soybean lecithin (B2 and B5) could not yield a dry powder form, whereas those with tween 80 or poloxamer 188 (B1, B3, B4, and B6) appeared as white to slightly yellowish coarse powders. The color of AASLMs was not

significantly affected by the type of surfactant. The physical appearances of the obtaining powders could be attributed to the unique characteristics of beeswax (Bogdanov, 2004). In addition, the pH values of all formulations ranged from 5.0 to 5.5 which were compatible with human skin (pH 4-6) (Klee *et al*., 2009; Lambers *et al*., 2006). The formulas B1, B3, B4 and B6 were selected for further study.

Table 1 The physicochemical characteristics of asiatic acid loaded in SLMs formulations.

Formula	Lipids (g)	Surfactants (g)			pH	Appearance	
	Beeswax	Tw80	L	P188		before freeze-drying	after freeze-drying
B1	10	3	-	-	5	Liquid/ Crystallization	White to light yellow coarse powder
B2	10	-	3	-	5	Liquid/ Crystallization	Light yellow, sticky lump
B3	10	-	-	3	5.5	Liquid/ Crystallization	White to light yellow coarse powder
B4	15	3	-	-	5	Liquid/ Crystallization	White to light yellow coarse powder
B5	15	-	3	-	5	Liquid/ Crystallization	Light yellow, sticky lump
B6	15	-	-	3	5.5	Liquid/ Crystallization	White to light yellow coarse powder

L = Lecithin, P188 = Poloxamer188, Tw80 = Tween80

Figure 1 The AASLMs prepared from 10% Beeswax: Tw80 (B1), 10% Beeswax: P188 (B3), 15% Beeswax: Tw80 (B4) and 15% Beeswax: P188 (B6).

3.2 Morphological analysis

The morphology of AASLM dispersions before freeze-drying and after redispersion of the freeze-dried powder observed under the optical microscope with a magnification of 100x was shown in Figure 2. In all the dispersions, the AASLM prepared from 10% or 15% beeswax with 3% poloxamer 188 (B3 and B6) had spherical particle shape (Figure 2(f) and (h)), whereas those prepared from 10% or 15% beeswax with 3% tween 80 (B1 and B4) seemed to have irregular particle shape (Figure 2(e) and (g)). This may be because P188 can effectively reduce the surface tension between the lipid phase and the aqueous phase during emulsification, resulting in a spherical particle shape. Moreover, the steric effect of P188 could prevent a droplet agglomeration (Hariyadi *et al*., 2022; Rosita *et al*., 2019) hence the AASLMs stabilized by P188 (B3 and B6) showed comparable particle shape before and after freeze-drying. On the contrary, tween 80 (Tw80) may not be able to effectively reduce the surface tension between the aqueous phase and the lipid phase during emulsification, resulting in irregular particle shape of AASLMs stabilized by Tw80. The results demonstrated that the type of surfactants used could affect the particle shape of the AASLMs.

The SEM images of formulas B3 and B6 powders were then subsequently characterized. It was found that the surface morphology of B3 and B6 powders was not spherical in shape (Figure 3). This finding was in agreement with previous studies which reported that the SLM powder produced by freeze-drying process had non-spherical and agglomerated morphology (Alihosseini *et al*., 2015; Mudrić *et al*., 2021; Owuor *et al*., 2017). The particle agglomeration has a positive effect on flow characteristic of particles and decreasing dust formation (Barbosa-Cánovas *et al*., 2005; Paucar *et al*., 2016).

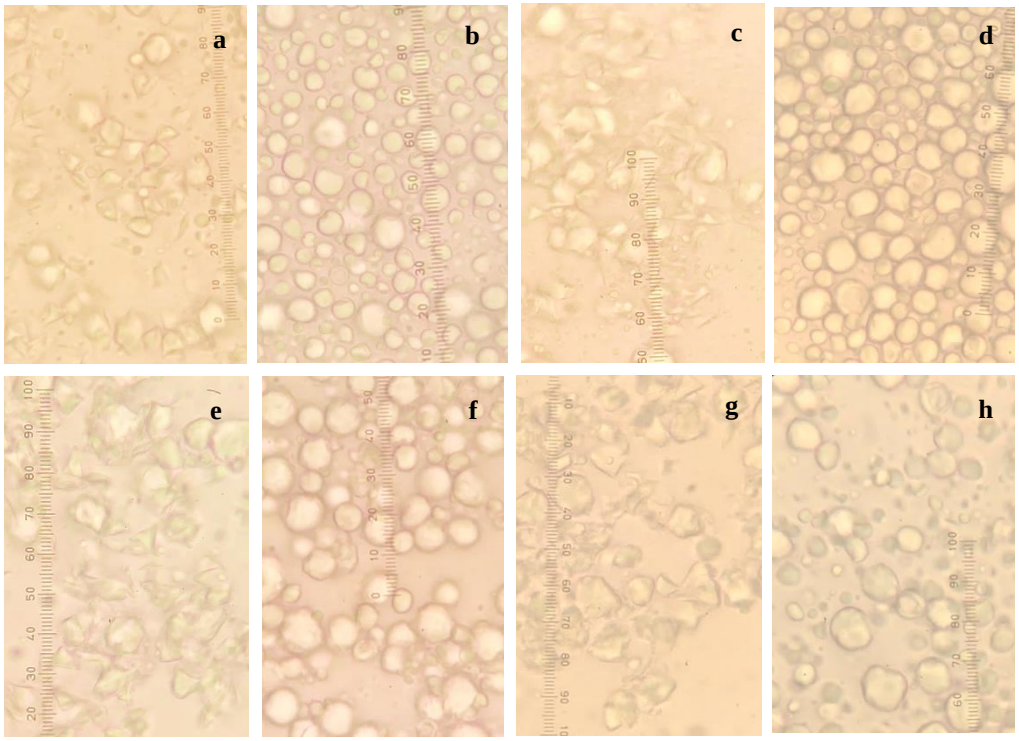

Figure 2 The optical microscope images of AASLMs formulations, before freeze-drying; (a) 10% Beeswax: Tw80 (B1), (b) 10% Beeswax: P188 (B3), (c) 15% Beeswax: Tw80 (B4), (d) 15% Beeswax: P188 (B6) and after freeze-drying; (e) 10% Beeswax: Tw80 (B1), (f) 10% Beeswax: P188 (B3), (g) 15% Beeswax: Tw80 (B4), (h) 15% Beeswax: P188 (B6).

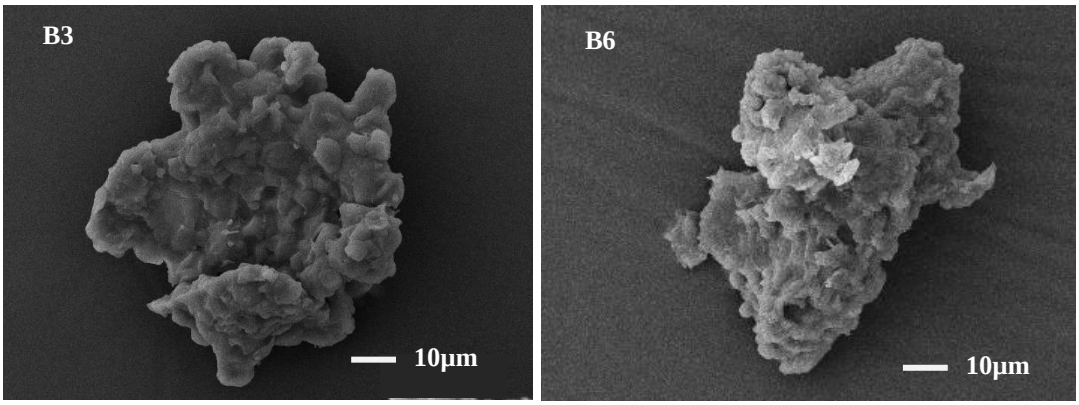

Figure 3 Scanning electron microscope images of AASLMs formulations; 10% Beeswax: P188 (B3) and 15% Beeswax: P188 (B6).

3.3 Particle size analysis

As shown in Table 2, the particle size of the AASLMs powder stabilized by Tw80 (B1, B4) was significantly larger than those stabilized by P188 (B3, B6). The stearic barrier provided by poloxamer may prevent an aggregation of SLM particles leading to a smaller particle size. The results suggested that the type of surfactants used had an effect on the particle size of the resultant AASLMs powder. In addition, there was no straight correlation between the size of SLMs and the amount of solid lipid used. The particle size of all formulations was in acceptable range (micron size) for topical application (Üner & Karaman, 2013).

Table 2 Particle size (µm) of obtaining AASLM powders.

Formula	Particle size (µm)
B1	38.86 ± 0.34*
B2	N/A
B3	14.15 ± 0.11*
B4	30.67 ± 0.27*
B5	N/A
B6	16.87 ± 0.15*

The * symbol represents significant difference ($p < 0.05$), N/A is not applicable.

3.4 Entrapment efficiency and active loading

The entrapment efficiency (EE) and active loading (AL) of resultant AASLM powders were shown in Figure 4 and 5, respectively. AASLMs stabilized by P188 (B3 and B6) gave higher %entrapment efficiency (%EE) and %active loading (%AL) than those stabilized by Tw80 (B1 and B4) ($p < 0.05$) This may be because P188 could immobilize the solid lipid system and improve solubility of poorly water soluble drug resulting in lower drug expulsion (Shah & Serajuddin, 2012). Previous study had also demonstrated that the solid lipid microparticle stabilized by poloxamer 188 had high entrapment efficiency of poorly water soluble substance (Hariyadi *et al*., 2022). The %labeled amount of all AASLMs formulations were in the range of 90.43±0.02 to 90.69±0.02%, which was considered acceptable for topical products (convention, 2004).

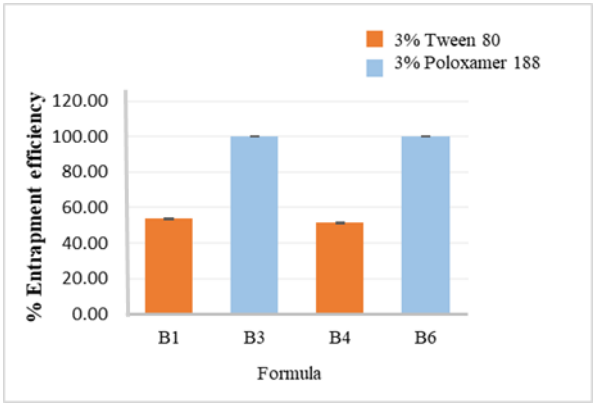

Figure 4 %Entrapment efficiency of asiatic acid solid lipid microparticles.

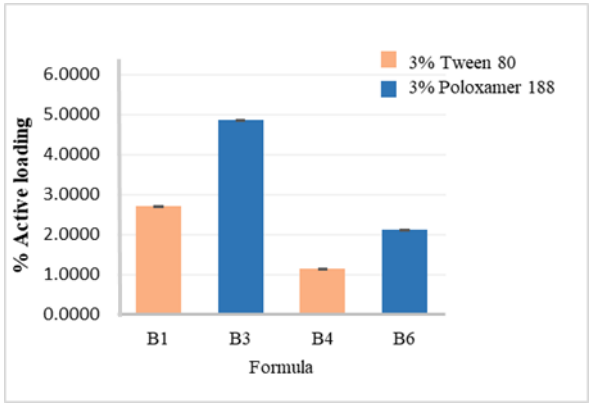

Figure 5 %Active loading of asiatic acid solid lipid microparticles.

Furthermore, the higher concentration of solid lipid (15%) used in B3 and B6 compared to those of B1 and B4 (10%) could have attributed to greater %EE and %AL. The results indicated that the amount of lipid used had an impact on the entrapment efficiency and active loading of AASLMs.

4. Conclusion

This study indicated that the type of surfactant used in AASLMs preparation could significantly affect the physicochemical properties including morphology, particle size, %EE and %AL of the resultant AASLMs, whereas the amount of solid lipid used could influence the entrapment efficiency and active loading of the AASLMs. In addition, it was identified that the AASLM prepared with poloxamer 188 has the greatest potential among the test formulations as a topical carrier for AA due to its high %EE and %AL. Further studies should be conducted to explore its utilization in cosmetics.

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Bioactive chemical constituents from the rhizome of black galingale

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Abstract

Krachai-Dam (KD), also known as black galingale (*Kaempferia parviflora*), is an herbaceous plant. The rhizomes of black galingale are the source of bioactive compounds with pharmacological effects, including anti-inflammatory and antivirus properties. The black galingale possesses intriguing cosmetic qualities, including antibacterial and anti-aging antioxidant capabilities. The solvent system was optimized using column chromatography to separate compounds A and B from the black galingale crude extract. The fundamental chemical components of the isolated substances from this study were identified utilizing FT-IR, GC-MS, ^{1}H-NMR and ^{13}C-NMR Spectroscopy methods. Antioxidant activity was present in all sample series. The FT-IR spectral data of the identified compound A showed a unique vibration band at 3470 cm^{-1}, 3068 cm^{-1}, and 1608 cm^{-1} corresponding to an OH stretching, CH stretching of an alkene (C=C-H), and C=C stretching. The GC-MS chromatogram showed *m/z* at 298 matching the molecular formula of $C_{17}H_{14}O_5$. The FT-IR spectral data of compound **B** was similar to that of compound **A,** and the m/z ion peak at 327 was indicated to be $C_{18}H_{16}O_6$. Moreover, the antioxidation properties of compounds **A** and **B** were examined by the ABTS assay at a concentration of 1 g/mL. The ABTS radical cation scavenging activity of both compounds were reported to be at 98.36% and 96.88% of the antioxidant activity of compound **A** and compound B, respectively. Compared to Trolox, where 238.11 ± 2.58 mM Trolox Equivalent (TE)/g sample and 233.5 ± 32.87 mM. The stable black galingale extract nanoemulsion was prepared using 10%w tween 80 as a surfactant. The particle size of the nanoemulsion was 27.63±0.86 nm.

Keywords: *Kaempferia parviflora*, Flavonoids, Zingiberaceae, Column Chromatography

1. Introduction

Black galingale (pronunciation) is a medicinal plant. Its scientific name is kaempferria parviflora Wall. Ex Baker, and it is a member of this plant family. Zingiberaceae is native to Thailand, Laos, Myanmar, India, and South Asia of China, and includes ginger, galangal, turmeric, sapphire, and white ginseng. It is common in Thailand's forests and at the foot of mountains. It is found primarily in Loei, Phitsanulok, Phetchabun Nan, and Kanchanaburi. Tak, Chiang Mai, and Chiang Rai are all popular tourist destinations. Ban Khek Noi, Khao Kho District, Phetchabun Province is home to the well-known Krachai Dam. Thailand's black ginger is of high quality and has many beneficial medicinal properties. According to Thai textbooks, black ginger is a type of herbal medicine that improves sexual function, helps to drive away wind, stimulates appetite, relieves heartburn, and relieves flatulence. It can also help to prevent skin aging caused by UV rays[1]. As a result, it is known as Thai ginseng. Currently,

black ginseng is processed into a variety of products. Dry black ginger, powdered black ginger, tea, black ginger wine, energy drinks, and so on[2].

Black galingale anatomy

KD is a plant that belongs to the same species as yellow galingale, which is a plant that is utilized in culinary applications. The useful part is the root or tuber that is underground in the soil. The height of the stem comes in at roughly 90 centimetres a solid core that is wrapped in a leaf sheath and located in the middle of the stem. The fragrance of the leaves is very enticing. The petiole emerges from the ground near the head, the leaves are seven to nine centimetres long, and the flowers are thirty to thirty-five centimetres in length. Rhizomes are characterized by their spherical shape and linear arrangement. Rhizome skin can range in color from very light brown to extremely dark brown. Black galingale rhizomes have an interior color that ranges from mild to dark purple. The many hues of the rhizome were used by growers and purchasers to classify the commodity and set pricing.

Phytochemical composition of Black galingale rhizome

Phytochemicals of black galingale extract were studied. Natural products including anthocyanins and flavonoids were reported [3]. In addition, phenolic compounds [4] with antimicrobial activity such as Borneol and Sylvestrene were present. Active ingredients belonging to flavonoids include 5,7-dimethoxyflavone (DMF), 5,7,4'-trimethoxyflavone (TMF), and 4'-pentamethoxyflavone (PMF) [5].

Black galingale in cosmetic

Polymethoxyflavones isolated from Black galingale rhizomes have potential anti-aging activities by promoting collagen, fibrillin, and hyaluronic acid synthesis under the skin and inhibiting reactive oxygen species (ROS) release and cellular senescence. Additionally, the flavonoid and terpene compounds from the rhizomes have antioxidant activity and showed inhibition of tartrate-resistant acid phosphatases (TRAP) with 16.97±1.02 to 64.67±2.76% and exhibited peroxyl radical scavenging capacity with 8.47±0.52 μM [2]. Also, *K. parviflora* has anti-inflammatory activity. The ethanol extract of *K. parviflora* inhibited PGE2 release with an IC50 value of 9.2 μg/ml [6].

2. Materials and Methods

2.1 Materials

2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 2,2'-diphenyl-1-picrylhydrazyl (DPPH), and Trolox were purchased from Sigma Chemical Co. Ethyl acetate All other chemicals used, including solvents, were of analytical grade. Black Galingale Rhizome was purchased from the Siang Adisorn market at Lom sak Distric, Phetchabun Province.

2.1 Preparation of samples

The air-dried black galingale rhizomes were collected from Phetchabun province. Cut into small pieces or grind finely. The ethyl acetate extract [7] was filtered and concentrated Under reduced pressure using a rotary evaporator.

2.2 Column Chromatography

Based on TLC analysis, the filtrated ethyl acetate extract (133.65 g) was chromatographed on a silica gel column (9.0 x 120 cm, silica gel, 100-200 mesh, 1500 g) and progressively rinsed with hexane/ethyl acetate (100:0, 98:2, 95:5, 90:10, 80:20, 70:30, 60:40, 50:50.

2.3 Spectroscopic analysis

Extracts of KD were subjected to thin layer chromatography (TLC) using solvent systems Hexane: Ethyl acetate (80:20). UV-Vis spectrophotometry (range 200-600 nm) was used to check the presence of flavonoids. Fine powder extract was mixed with Potassium bromide (FT-IR grade) in a ratio of 1 mg:10 mg. The powdered sample was loaded in Fourier Transform Infrared Spectrometer (FT-IR) (Shimadzu, IR affinity 1S) to detect the characteristic peaks and functional groups in the range of 400-4000 cm^{-1}. GC-MS with helium carrier gas at 36 cm/s, were used in the GC/MS experiments. experiments were carried out. At 50 cm/s, GC with hydrogen carrier. All injections were carried out in the split mode with a 20 split ratio The temperature of the injector. The detector or interface temperature

was 310°C, while the ambient temperature was 280°C. At 310°C, retention measurements were taken isothermally. Standard hydrocarbons range from C20 to C40-[8]. ^{1}H NMR and ^{13}C NMR spectra were recorded in MeOH-d_4 at 25 °C on a Bruker AMX 300 spectrometer (Karlsruhe, Germany; ^{1}H 400 MHz; ^{13}C 400 MHz)- were performed on a Bruker Ascend 400 spectrometer. Chemical shifts (δ) are reported in ppm relative to the residual solvent signals.

2.4 Black galingale rhizome extract nanoemulsion preparation

Black galingate rhizome extract was dissolved in DMDiol (80% Methylpropanediol 20% Decane-1,2-diol) as oil phase. Tween 80 (T80) as surfactant was mixed in aqueous phase with various ratios (2.5 – 10 %w). Aqueous phase was poured into oil phase using homogenizer at 8000 rpm for 30 min. Black galingale rhizome nanoemulsion was keep in room temperature.

2.5 Antioxidant activity

2.5.1 2,2-diphenyl-1-picryhydrazyl (DPPH) radical scavenging activity

The extracts' DPPH free radical scavenging activity was tested following our earlier findings [9]. In brief, a DPPH (0.1 mM) solution in methanol was produced, and 1 mL was applied to varied amounts of extract sample (1.0 mL). The mixture was rapidly mixed and incubated in the dark for 30 minutes. At 517 nm, the absorbance was measured by UV-Vis In the control, methanol was used in place of the sample. The inhibitory ratio (%) was obtained using the equation:

$$\text{Inhibition percentage (I\%)} = \frac{\text{Abscontrol} - \text{Abssample}}{\text{Abscontrol}} \times 100$$

All determinations were made in triplicate and were determined to be repeatable within the range

2.5.2 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) radical cation decolorization assay

The ABTS test was conducted based on prior conclusions [10]. In ultrapure water, an ABTS solution (7 mM) was combined with potassium persulfate (2.45 mM). The combination was kept in the dark for 12 hours before application. The addition of 500 L extract samples of various strengths to appropriately diluted ABTS solutions was completed by a 6-minute measurement of the absorbance at 734 nm. Methanol was utilized as the control in place of the sample. Following is how the inhibition percentage (I%) was calculated:

$$\text{Inhibition percentage (I\%)} = \frac{\text{Abscontrol} - \text{Abssample}}{\text{Abscontrol}} \times 100$$

The experiments were repeated three times with comparable results.

3. Results and discussion

3.1 Physical characteristics and spectral data

The chemical structures of two isolated flavones were determined using UV-Vis, FT-IR, GC, and ^{1}H-NMR, ^{13}C-NMR spectroscopy. The flavone structures were known compounds previously isolated. In this study, their antioxidant properties were determined.

3-Hydroxy-5,7-dimethoxyflavone (A): Yellow amorphous powder, mp. 166-169 °C, GC-MS *m/z* 298, RT (min) 38.11; UV (EAC, λ_{max}): 274, 298, 350 nm; IR (KBr, υ) 3072, 2843, 2360, 1605, 1155, 830, 766 cm^{-1}: ^{1}H-NMR ($CDCl_3$, 400 MHz, δ_H ppm) and ^{13}C-NMR ($CDCl_3$, 400 MHz, δ_C ppm) see Table 1.

5-Hydroxy-3,7,4'-trimethoxyflavone (B): Yellow amorphous powder mp. 145-147 °C, GC-MS 329 *m/z*, RT (min) 40.47; UV (EAC, λ_{max}): 269, 319, 348 nm; IR (KBr, υ) 3434, 3072, 2843, 1623, 1148, 833 cm^{-1}:^{1}H-NMR ($CDCl_3$, 400 MHz, δ_H ppm) and ^{13}C-NMR ($CDCl_3$, 400 MHz, δ_C ppm) see Table 1.

3.2 Antioxidant activity

The antioxidant activity of natural compounds was evaluated using DPPH (2,2-diphenhydramine-

Table 1 ^{1}H NMR and ^{13}C-NMR data of isolated compound

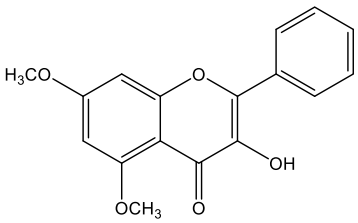
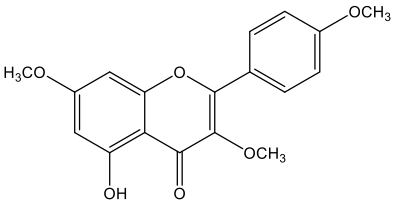
Compound	Characterizations
 **3-Hydroxy-5,7-dimethoxyflavone**	**^{1}H NMR($CDCl_3$):** δ 3.87(6H, S, OCH3) 3.92(3H, S, OCH3), 6.36(1H, d), 6.45(1H, d),7.51 (3H, m), 8.07(2H, m) 12.59 (1H, S, OH); **^{13}C-NMR($CDCl_3$):** δ 55.96, 60.53, 92.34, 98.09, 106.33, 128.53, 128.75, 130.61, 131.08, 139.82, 156.05, 157.04, 162.17, 165.71,175.92 179.09
 **5-Hydroxy-3,7,4'-trimethoxyflavone**	**^{1}H NMR($CDCl_3$):** δ 3.86 (6H, S, OCH3) 3.87(3H, S, OCH3), 6.35(1H, d), 6.44(1H, d),7.03(2H, d), 8.09(2H, d) 12.73(1H, S, OH); **^{13}C-NMR($CDCl_3$):** δ 55.62, 55.84, 60.21, 92.13, 97.84, 105.44, 114.52, 125.64, 130.29, 138.75, 155.81, 156.11, 161.20, 161.92, 166.40, 178.7

Table 2 DPPH assays were used to evaluate antioxidant activity by comparing the standard concentration of Trolox.

Compound	Concentration of the extract (mM)	%DPPH free radical inhibition	Standard concentration of Trolox	TEAC (mg Trolox/g Extract)
A	1.0	65.43	0.23	237.86±2.10
	2.0	72.69	0.26	131.49±1.21
B	1.0	29.49	0.11	115.32±1.50
	2.0	48.43	0.18	90.03±1.28

1-picrylhydrazy) and ABTS (2,2,-azino-bis (3-ethylbenzhiazoline-6-sulphonic acid)) assays. DPPH scavenging assays are simple assays for evaluating the antioxidant capacity of the compound [11]. The antioxidant activity of the ethyl acetate extract of KD was determined by its antioxidants' ability to inhibit oxidation. All fractions were evaluated for their antioxidant activities at the concentrations of 0.0001 µg/mL and 2.0 µg/mL. The ethyl acetate extract and a fraction showed antioxidant activities. The antioxidant activity of the ethyl acetate extract of KD extracts is reported in Tables 2 and 3.

3.3 Black galingale rhizome extract nanoemulsion

Tween 80 as a surfactant formulated black galingale rhizome nanoemulsion at 2.5, 5.0, 7.5 and 10 % by weight, respectively. The particle size, zeta-potential and PDI were evaluated by Zetasizer. The results show that 10.0%w of Tween 80, can provide appropriate black galingale rhizome extract nanoemulsion without phase separation. The particle size was at 27.63±0.86 nm and zeta-potential was at -46.2±0.26 mV, which indicates good stability. The droplet polydispersity index (PDI) of black galingale extract nanoemulsion was found to be at 0.22±0.035, which is below the 0.3 mark, indicating a narrow size distribution.

4. Conclusions

The ethyl acetate crude extract of KD from Phetchabun was corrected and isolated by column chromatography by which it yielded two known flavonoid compounds. These are 3-hydroxy-5,7-dimethoxyflavone (A) and 5-hydroxy-3,7,4'-trimethoxyflavone (B), respectively.

Compounds A and B when tested for antioxidant activity by ABTS method showed better antioxidant activity than the DPPH method. Furthermore, when compared with the Trolox, %DPPH, %ABTS of Compound A and B, the results show antioxidant tendency with higher free radicals. When increasing concentrations of compounds A and B, TEAC have a tendency to decrease. The black galingale rhizome extract nanoemulsion was prepared utlising the Tween 80 as a surfactant. It was found that at 10% tween, the surfactant was enough to stabilize the oil phase and provide good stability as -46.2±0.26 mV of zeta-potential value.

Table 3 ABTS assays were used to evaluate antioxidant activity by comparing the standard concentration of Trolox

Compound	Concentration of the extract (mM)	%ABTS free radical inhibition	Standard concentration of Trolox	TEAC (mg Trolox/g Extract)
A	1.0	98.36	0.23	238.11±2.23
	2.0	98.97	0.24	119.05±1.19
B	1.0	96.88	0.23	233.53±2.87
	2.0	98.65	0.24	116.74±2.23

Table 4 Particle size, Zeta potential (ZP) and polydispersity index (PDI) of black galingale rhizome extract nanoemulsion

Formulation	T80 (%w)	Appearance	Size (nm)	ZP (mV)	PDI
F1	2.5	Miscible	-		
F2	5.0	Miscible	-		
F3	7.5	Miscible	-		
F4	10.0	Immiscible	27.63±0.86	-46.2±0.26	0.22±0.035

5. Acknowledgement

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Identification of phytochemicals and antioxidants in ethanolic extracts of *Dialium cochinchinense* using LC-ESI-MS coupled with DPPH assay

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Abstract

Dialium cochinchinense is a plant in family Fabaceae and the genus *Dialium*. It is an edible fruit containing vitamins, minerals, and antioxidants. The fruit is composed of three main parts including the mesocarp (pulp), exocarp (skin or peel), and seed. This study was aimed to evaluate antioxidant activity of *D. cochinchinense* fruit extracts by 2,2-diphenyl-picrylhydrazyl (DPPH) assay and identify phytochemicals by liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS). The exocarp extract (EEE), mesocarp extract (MEE), seed extract (SEE), and whole fruit extract (WFE) were macerated in 95% (v/v) ethanol by shaking at room temperature for 48 h. The highest extraction yield of crude extracts was observed in WFE followed by MEE, EEE, and SEE at 32.6±0.6% (w/w), 22.4±1.5% (w/w), 13.0±0.4% (w/w), and 7.8±0.0% (w/w), respectively. The IC50 values of the extracts were 0.024 mg/mL in SEE, 0.544 mg/mL in EEE, 0.569 mg/mL in WFE, and more than 50 mg/mL in MEE. Due to the strongest antioxidant activity, SEE (1000 ppm) was taken for further phytochemical analysis by LC-ESI-MS. Based on its mass spectra and MS/MS fragment masses, a group of vitamin D derivatives, Lippioside II, and Solanocapsine were expected to provide antioxidant activity. The results can be concluded that the ethanolic seed extract of *D. cochinchinense* could provide potential benefits in nutraceutical, cosmeceutical, and beauty applications.

Keywords: Antioxidant; *Dialium cochinchinense*; DPPH; LC-MS/MS; Phytochemicals

1. Introduction

Oxidative stress can be caused by endogenous factors such as reactive oxygen species (ROS) and exogenous factors such as UV radiation, smoking, pollution, organic solvents, pesticides. Free radicals produced among the oxidative stress can damage nucleic acids, proteins, enzymes, and other small molecules causing loss of structure and function (González-Palma *et al*., 2016). To prevent the damages, the human body has a naturally antioxidant enzyme system as well as receives the diet with antioxidants for reducing free radicals. Antioxidants can neutralize free radicals by donating one electron to an unstable oxygen atom of free radicals. The antioxidants have been widely used for cosmetic and beauty application. Many antioxidants are beneficial for improving the quality of some skin appearance, particularly aged skin, because they can prevent the breakdown of skin cells and wrinkles caused by free radicals. As a previous study, the results showed that a direct application of antioxidant cream to the skin might slow down the aging process (Ahmed *et al.*, 2015).

Natural antioxidant substances have been increasing for many applications because they are expected to be safer than synthetic antioxidants (Pokorný, 2007). Plant or botanical source is one of the natural sources that have been studied for discovering new antioxidants. *Dialium cochinchinense* is a fruit-bearing tree belonging to the Fabaceae family. The plant is native to Sabah, Sarawak and found

widely in Cambodia, Laos, Malaysia, Myanmar, Vietnam, and Thailand (Schmidt and Nguyen, 2005). Fruits of *D. cochinchinense* are usually produced between the months of March and May but may be earlier and sometimes persist longer (Lasekan and See, 2015). The fruits are enclosed in a brittle shell. The seed is embedded in a dry brownish sweet slightly sour edible pulp. The bioactivity of D. *cochinchinese* has been rarely. However, there was much research in *D. guineense*, another species of the *Dialium* genus. The dichloromethane fraction of *D. guineense* fruit coat exerted wound-healing and antimicrobial properties (Okeke *et al.*, 2016). Moreover, the phenolic extract of *D. guineense* pulp enhanced reactive oxygen species detoxification in aflatoxin hepatocarcinogenesis (Adeleye *et al.*, 2014). Moreover, the bark extract of *D. guineense* exhibited antibacterial activity while the leaf extracts had antioxidant and antimicrobial activities (Gideon *et al*., 2013). According to the biological properties of *D. guineense*, it is believed that *D. cochinchinese* may be possess many properties, especially antioxidant activity.

Therefore, the objectives of this study were to determine the antioxidant activities of *Dialium cochinchinense* fruit (exocarp, mesocarp and seed) and investigate phytochemical compounds by liquid chromatography–mass spectrometry (LC-MS/MS) technique.

2. Materials and Methods

2.1 Plant preparation

Dialium cochinchinense fruits were purchased from a local farm in Yarang, Pattani, Thailand. Healthy and uninfected fruits of *D. cochinchinense* were carefully selected. The exocarp (peel), mesocarp (pulp), and seed of the fruits were separated manually (Figure 1). The fruit parts were dried in the drying oven at 45 C° for 48 h. The dried parts were ground into a fine powder and kept in plastic containers at room temperature until use.

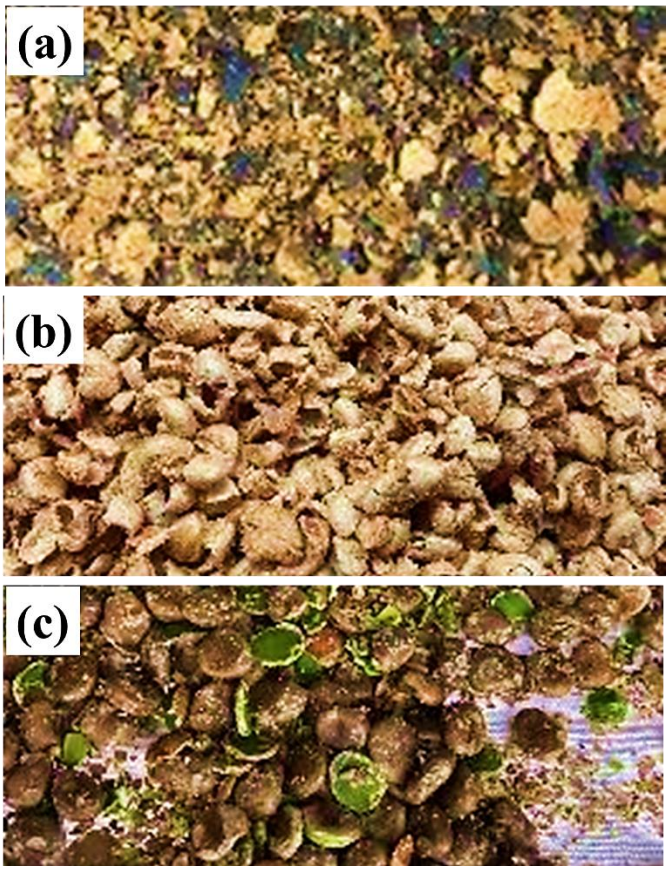

Figure 1 Parts used of *D. cochinchinese* fruits including exocarp (a), mesocarp (b), and seed (c).

2.2 Extraction of the original Dialium cochinchinense fruit parts

Dried powder (25 g) of each fruit parts were macerated in absolute ethanol (250 mL) at room temperature for 48 h. The extraction procedure was repeated three times. The supernatants were collected and filtered by Whatman No.1 filter paper. The remaining ethanol in filtered extracts was removed by a rotary evaporator. The extracts were yielded via the percentage yield and then stored in -20 °C for the further test (Olajubu *et al*., 2012).

2.3 DPPH radical scavenging assay

The antioxidant activity of *D. cochinchinense* extracts was performed by DPPH radical scavenging assay (Thitilertdecha *et al*., 2008). The extracts with various concentration were prepared by propylene glycol, and 0.1 mM DPPH solution in absolute methanol was used for the evaluation. Afterward, to various concentration of test samples (10 μL) 0.1 mM DPPH methanol solution (100 μL) was added. After 30 min of incubation in the dark at room temperature, the absorbance was measured at the wavelength of 515 nm using a micro-plate reader. The IC50 values of sample tests were calculated. Ascorbic acid was used as a standard for comparison.

2.4 Liquid chromatography–electrospray-ionization mass spectrometry (LC-MS/MS)

Seed extract (1 mg) was dissolved in 1 mL of absolute methanol. The extract sample was filtered through 0.2 microns syringe to small vials. Phytochemicals in the seed extract were investigated by LC-ESI-MS. The liquid chromatography was performed on UHPLC Binary pump, Agilent 1290 Infinity ii LC system by Mr. Poramet Nachalam, who is an expert technician at Advanced Analytical Equipment Service at the Central Laboratory for Research, Mae Fah Luang University. The extract samples were separated on XBridge C18, 2.5 um, 2.1*100 mm column at 35 °C. The mobile phase A was 0.1 % formic acid in water and mobile phase B was 0.1 % formic acid in acetonitrile. The flow rate was 0.2 ml/min. The information of 6545B QTOF/MS system Interface: Dual agilent jetstream ESI (Dual AJS ESI). MS/MS was performed in both positive (121.0508,922.0097m/z), and negative (112.9855,1033.9881) modes and scanned at the mass range of m/z 50-1100 amu scan rate: spectra/second. Control and data handling of the instrument was achieved by the same software as analytical HPLC. Data analysis software was performed by Mass hunter LC/MS Data Acquisition for 6200 series TOF/6500 Series Q-TOF Version B.08.00 Build 8.00.8058.0 and Masshunter Qualitative analysis Version B.08.00.

3. Results and Discussion

3.1 Yields of extracts

The extraction yields of exocarp extract (EEE), mesocarp extract (MEE), seed extract (SEE), and whole fruit extract (WFE) were shown in Table 1. AEE resulted in the highest extraction yields were showed at 32.6%, followed by EEE showed at 13%, MEE showed at 22.4% and seed extract showed at 7.8% Among the separated fruit parts, the percentage of extraction yield mesocarp was the highest, which is expected to contain phenolic, δ-tocopherol, d-glucose and sucrose (Osman *et al.*, 2018).

Table 1 Extraction yields of *D. cochinchinese* parts obtained by ethanol extraction.

Extracts	%Extraction yield ± SD
Exocarp extract (EEE)	13.0±0.4
Mesocarp extract (MEE)	22.4±1.5
Seed extract (SEE)	7.8±0.0
Whole fruit extract (WFE)	32.6±0.6

Triplication of each extract was used to calculate % extraction yield.

3.2. Antioxidant activity of *D. cochinchinese* extracts

When a solution of DPPH is mixed with a substance that can donate a hydrogen atom, the violet color will change (Thitilertdecha *et al.*, 2008). Antioxidant activity results according to analyses of DPPH assay for *D. cochinchinense* extracts was presented in Table 2.

Table 2 Antioxidant activity of *D. cochinchinese* extracts evaluated by DPPH method.

Extract	IC_{50}(mg/ml)
Mesocarp extract (MEE)	>50
Exocarp extract (EEE)	0.544
Seed extract (SEE)	0.024
Whole fruit extract (WFE)	0.569
ascorbic acid	0.003

*Tests were accomplished in triple and explicit mean; IC_{50} explicit in mg/ml

The IC_{50} value indicates the amount of antioxidant activity. The ethanol extracts showed remarkable antioxidant activities. The IC_{50} values of MEE, EEE, SEE and WFE were more than 50 mg/ml, 0.544 mg/ml, 0.024 mg/ml, and 0.569 mg/ml, respectively. As a result, SEE had the highest antioxidant activity when compared with other extracts. In the same way, previous studies on the antioxidant activity of *D. cochinchinense* fruit had been conducted. The seed methanol fraction showed the strongest antioxidant activity including total antioxidant capacity and DPPH radical scavenging activity than the other fractions. In addition, the exocarp dichloromethane fraction was the discriminating fraction by having linoleic acid peroxidation inhibition. A total of thirty-eight metabolites were detected in derivatized exocarp dichloromethane and seed methanol fractions with distinctive classes of phenolics and amino acids, respectively. Bioautography-guided fractionation of exocarp dichloromethane fraction afforded five antioxidant-enriched subfractions with four others detected phenolics. The results showed the antioxidant properties of *D. cochinchinense* fruit (Osman *et al*., 2018).

3.3. Liquid chromatography–electrospray-ionization mass spectrometry (LC-MS/MS) assay

The results of LC-MS/MS analysis of SEE were shown in (Table 3) The compounds in SEE were analyzed by LC-MS/MS. As shown in Table 3, the major fifteen compounds were identified. A group of vitamin D derivatives, Lippioside II, and Solanocapsine in SEE were found and expected to provide antioxidant activities. From the literature review, the antioxidant capacity of some vitamins were known, such as vitamin C and vitamin E, and even vitamin D (Tagliaferri *et al.*, 2019). The 25-Hydroxyvitamin D3 was a vitamin D derivative and could promote antioxidant capacity in vivo model (Zhou *et al.*, 2022). Lippioside II was found as a minor compound in the extract of *Lippia citriodora* leaves evaluated by HPLC-ESI-TOF-MS. L. citriodora has been demonstrated to possess many phytochemicals which provide benefits to human health acting as antioxidants or anti-obesogenics (Leyva-Jiménez *et al*., 2020). Solanocapsine was found in plants belonging to the Solanaceae family including Solanum incanum L., S. schimperianum Hochst, S. nigrum L., Physalis lagascae Roem. & Schult. and Withania somnifera (L) Dunal. The methanolic leaf extracts and steroidal glycoalkaloids fractions (SGAFs) of these plant species were taken for antibacterial, antiproliferative and antioxidant activities. The result of the antioxidant investigation was shown that solanopubamine and solanocapsine were suggested to provide antioxidant activity (Fadl Almoulah *et al*., 2017).

4.Conclusion

In conclusion, *D. cochinchinense* extracts provided antioxidant activity against DPPH radicals. Among these extracts, SEE showed the highest antioxidant activity. Based on its mass spectra and MS/MS fragment masses, a group of vitamin D derivatives, Lippioside II, and Solanocapsine in SEE were expected to provide the antioxidant activity. The results can be concluded that the ethanolic seed extract of *D. cochinchinense* could provide potential benefits in nutraceutical, cosmeceutical, and beauty applications.

5. Acknowledgements

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Table 3 The compounds in SEE were analyzed by LC-MS/MS.

No	m/z	RT	Ionization mode	Ion Species	MS/MS fragment masses	Molecular Formula	Mass	putative compound
1	104.108	19.932	positive	(M+H)+	70.0651 57.0703	$C_{16}H_{35}NO_2$	273.2686	Hexadecasphinganine,C16 Sphinganine
2	123.093	26.577	positive	(M+H)+	67.0418	$C_7H_{10}N_2$	122.0854	4-Amino-N-methylaniline,N-Methylpyridine-4-methylamine,Trimethylpyrazine
3	130.159	20.042	positive	(M+H)+	513.3402 514.3433	$C_{30}H_{50}O_3S$	490.3523	26,27-diethyl-1?,25-dihydroxy-22- thia-20-epivitamin D3 / 26,27- diethyl-1?,25-dihydroxy-22-thia20-epicholecalciferol,26,27-diethyl-1?,25-dihydroxy-22-thiavitamin D3 / 26,27-diethyl 1?,25-dihydroxy-22-thiacholecalciferol
4	274.274	20.354	positive	(M+H)+	57.0701 70.0650	$C_{16}H_{35}NO_2$	273.2681	Hexadecasphinganine,C16 Sphinganine
5	274.275	0.656	negative	(M-H)-	89.0245	$C_{24}H_{44}O_{22}$	684.2327	Lippioside II
6	274.276	19.920	positive	(M+NH4)+	601.3926	$C_{24}H_{45}N_{11}O_6$	583.3596	L-Valylglycyl-N~5~- (diaminomethylidene)-L-ornithylL.-prolyl-N~5~- (diaminomethylidene)-L-ornithine
7	311.169	20.062	positive	(M+NH4)+	171.1382 57.0703	$C_{30}H_{50}O_3S$	490.3515	26,27-diethyl-1?,25-dihydroxy-22- thia-20-epivitamin D3 / 26,27- diethyl-1?,25-dihydroxy-22-thia20-epicholecalc
8	434.172	20.440	positive	(M+H)+	211.1228	$C_{24}H_{23}N_3O_5$	433.1650	JZL 195
9	453.344	0.672	positive	(M+H)+	203.0529	$C_5H_{13}NO$	103.1003	2-Propoxyethylamine,N,N-Dimethyl-2-methoxyethylamine,N-(2-Methoxyethyl)ethylamine
10	469.314	20.766	positive	(M+H)+	58.0655	$C_{16}H_{35}NO_2$	273.2670	Hexadecasphinganine,C16 Sphinganine
11	508.385	26.287	negative	(M-H)-	183.012	$C_{17}H_{28}O_3S$	312.1760	Marshmine,Romucosine C,Promucosine
12	513.342	20.141	positive	(M+H)+	469.3192	$C_{27}H_{42}F_2O_4$	468.3070	(6RS)-6,19-epidioxy-24,24- difluoro-25-hydroxy-6,19-dihydrovitamin D3 / (6RS)-6,19- epidioxy-24,24-difluoro-25- hydroxy-6,19- dihydrocholecalciferol
13	601.393	9.757	positive	(M+Na)+	100.1122	$C_{27}H_{46}N_2O_2$	430.3545	Solanocapsine
14	683.226	27.015	positive	(M+NH4)+	62.9293	C_8H_{16}	112.1253	1-Hexene, 5,5-dimethyl-,1-Butyl-2-methylcyclopropane,3-Hexene, 2,2-dimethyl-
15	980.766	20.981	negative	(M-H)-	61.9888	$C_{32}HCl_3N_2O_{29}$	981.7731	Ala Phe Ala Arg

6. References

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Bioactive compounds and antioxidant activities of *Zingiber officinale* extracts for cosmetic applications

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Abstract

The research's purpose was to investigate the bioactive compounds and antioxidant activities of *Zingiber officinale* extract for cosmetic applications. The whole fresh ginger rhizome without the peel was used to extract using a sonication technique in a sonication bath at 25℃ for 60 minutes with 70% (v/v) and 95% (v/v) ethanol solvent (EtOH). The extracts were taken for evaluation of the bioactive compounds by total phenolic (TPC), flavonoid (TFC), and proanthocyanidin (TPAC) contents and antioxidant activities assayed by DPPH- and ABTS-radical scavenging activities and ferric-reducing antioxidant power (FRAP). The results showed that 70% EtOH extraction provided a maximum yield of 3.95±0.23%, whereas the extraction with 95% EtOH yielded 1.80±0.20%. For bioactive determination results, the TPC of 95% EtOH extract (408.83±1.54 mg GAE/g extract) was higher than that of 70% EtOH extract 260.15±2.45 mg GAE/g extract. The TFC and TPAC of 70% EtOH were 44.59±0.10 mg QE/g extract and 353.94±35.76 mg CE/g extract, while those of 95% EtOH extract were 40.76±0.26 mg QE/g extract and 329.88±3.30 mg CE/g extract, respectively. The trend of the antioxidant activity of the ginger extract with DPPH and FRAPS methods were similar to results of TPC. The 95% EtOH extract showed the highest antioxidant activities against DPPH and FRAP radicals at 902.04±0.70 and 1893.13±5.49 mg TEAC/g extract, while those of the 70% EtOH extract were 844.36±1.11 and 1388.11±65.51 mg TEAC/g extract, respectively. The antioxidant activity of 70% EtOH extract against the ABTS radical (650.14±1.86) was greater than that of 95% EtOH extract (616.55±10.02 mg TEAC/ g extract). The results suggested that the ethanolic extracts of *Zingiber officinale* can be used as an alternative source of natural antioxidants for cosmetic applications.

Keywords: Antioxidant Activity; Flavonoid; Phenolic; Sonication; *Zingiber officinale*

1. Introduction

Zingiber officinale, commonly known as ginger, belongs to the Zingerberaceae family, which has been widely used in the fields of cooking and medicine since ancient times. (Barceloux, 2008). Small molecules and a significant amount of essential oils were found in the ginger, as well as gingerols and shogols, due to its ability to stimulate blood circulation and have a warming effect; these are major components of the phenolics, which have antioxidant properties (Liu et al., 2019), so that *Z. officinale* is an important source for active ingredients in antiaging cosmetics.

Many studies have been done to find the best way to increase its performance and yield. So that benefits can be gained from the extraction method, the selection of natural plant extracts depends on the types of compounds and other conditions like the temperature, the solvents, the timing, etc. Some research reported that mixing extraction techniques provided the highest phenolic content and antioxidants (Gunathilake and Rupasinghe, 2014); however, the long thermal treatment had an impact

on the results, the price, and the performance components. Therefore, ultrasonic-assisted extraction is a substitute technique that enhances compounds, boosts yield, takes the shortest time, and is inexpensive (Vinatoru, 2001).

Furthermore, several studies have shown that the extraction method can alter the antioxidant activity and total phenolic content in the extracts (Chan, Lim, & Omar, 2007; Ding et al., 2012; Sikora, Cieslik, Leszcznska, Filipiak-Florkiewicz, & Pisulewski, 2008). Hsiang-yu (2014) reported that the results of bioactive components and antioxidant properties were comparable in two different plant species by using different extraction solvents between aqueous and ethanolic extracts; currently, the information on using different solvent concentrations between ethanolic extracts at 70% and 95% is unknown. Therefore, the objective of this study was to compare their total bioactive compounds and antioxidant properties in *Z. officinale* extracts using two different extraction concentrate solvents in a sonication bath to promote the utilization of botanical plants by turning academic research into commercial products for researchers, entrepreneurs, and people who are interested in natural plants.

2. Materials and Methods

2.1 Chemicals and reagents

All the reagents and solvents used were of analytical grade: Folin-Ciocalteu, Quercetin, Potassium Acetate, Catechin, Vanillin, DPPH (1,1-diphenyl-2-picrylhydrazyl), Trolox, Tptz, Sodium Phosphate Monobasic, Potassium Persulphate (k2S2O2), and ABTS (2,2-azinobis 3-ethyl-benzothiazoline-6-sulf) were purchased from Sigma Aldrich Chemistry Co.,Ltd. DMSO was purchased from CARLO ERBA Reagents, France. Ferric chloride, Gallic acid, Sodium carbonate (Na2Co3) were purchased from Merck. Aluminum Chloride (ALCL3) and Methanol were purchased from QREC, New Zealand. Sodium acetate was purchased from Loba Chemie Pvt. Ltd., India. Na2HPO4 was purchased from RCI Labscan Limited, Thailand.

2.2 Material Preparation

The fresh ginger rhizomes (*Zingiber officinale*) were obtained at the local market in Bangkok, Thailand, in January 2021. The samples were rinsed, and their peels were removed. The rhizomes were cut into small pieces (2.0 to 5.0 mm). All samples were dried in a hot air oven (Memmert, UF110, Germany) at 50 °C until their weight was constant. The dried samples were milled into powder and kept at room temperature.

2.3 Sonication extraction of bioactive compounds

The extraction used a sonication technique. Two different solvents were used to extract *Zingiber officinale* at a ratio of 1:10 (w/v): 70% and 95% ethanol. The mixture was sonicated in a sonication bath at 25 °C for 60 minutes at 45 kHz (Crest Ultrasonics, 690 DAE, Malaysia). The extracts were filtered through Whatman No. 1 filter papers. Solvents were removed using the rotary evaporator at 50 °C (Heidolph, Germany). Crude extracts were stored at 4 °C.

2.4 Total phenolic content (*TPC*)

TPC was measured according to Thitipramote *et al*. (2016; 2022) with a slight modification. Briefly, 20 µL of *Z. officinale* extract was reacted with 100 µL of 0.2 M Folin-Ciocalteu reagent and 80 µL of 7.5% Na_2CO_3. The mixture was incubated for 30 minutes at room temperature. The absorbance was measured at 765 nm using a microplate reader (Biotek, Epoch, USA). Gallic acid was used as a reference standard. The results were expressed as mg gallic acid equivalents (GAE)/g extract.

2.5 Total Flavonoid content (*TFC*)

TFC was measured according to Thitipramote *et al*. (2016) with a slight modification. Briefly, 25 µL of *Z. officinale* extract was mixed with 75 µL of ethanol, 5 µL of 10% (w/v) $AlCl_3$, 5 µL of 1 M CH_3 COOK, and 140 µL of DI water, respectively. The mixture was incubated for 30 minutes at room temperature. The absorbance was measured at 415 nm. Quercetin was used as a reference standard. The results were expressed as mg gallic acid equivalents (QE)/g extract.

2.6 Total proanthocyanidin content (*TPAC*)

TPAC was determined according to Thitipramote *et al*. (2016). Catechin (standard) (20 µL) was added to 50 µL of 1% vanillin (v/v) and 50 µL of 25% H_2SO_4 in methanol. The mixture was incubated in the dark for 30 minutes at room temperature. The absorbance was measured at 500 nm. The results were expressed as mg catechin equivalents (CE)/g extract.

2.7 DPPH radical scavenging activity (*DPPH*)

DPPH radical scavenging activity was performed as previously described (Thitipramote *et al*., 2016). Briefly, 10 µL of the sample was added to 190 µL of DPPH reagent. The reaction mixture performed satisfactorily in the dark for 30 minutes at room temperature. The absorbance was measured at 515 nm. As a reference standard, the Trolox solution was used. The percentage (%) of inhibition was calculated using the following formula:

$$\% \text{ inhibition} = \frac{(Abs\ control - Abs\ extract)}{Abs\ control}\ x\ 100$$

The results were expressed as mg Trolox equivalent antioxidant capacity (TEAC)/g extract.

2.8 ABTS radical scavenging activity (*ABTS)*

ABTS radical scavenging activity was performed as previously described (Thitipramote *et al*., 2016). The ABTS solution was freshly prepared by mixing 7.0 mM ABTS with 2.45 mM K2S2O8 (v/v). Then, the ABTS solution was diluted with 50 mM phosphate buffer (pH 7) at a ratio of 1:20 (v/v). For analysis, 10 µL of the sample was added to 190 µL of the diluted ABTS solution. The reaction was performed in the dark for 15 minutes at room temperature. The absorbance was measured at 734 nm. The Trolox solution was used as a reference standard. The percentage (%) of inhibition was calculated as mentioned above. The results were reported as Trolox equivalent antioxidant capacity (TEAC)/g extract.

2.9 Ferric reducing antioxidant power (*FRAP*)

FRAP was measured as previously described (Thitipramote *et al*., 2016). Firstly, FRAP solution was freshly prepared by mixing 1 mL of TPTZ solution (in 40 mM HCI), 1 mL of 20 mM ferric chloride solution, and 10 mL of 0.3 M acetate buffer (pH 3.6). For analysis, 10 µL of the samples were mixed with 190 µL of FRAP solution. The mixture was incubated in the dark for 15 minutes at room temperature. The absorbance was measured at 593 nm using a microplate reader. Trolox was used as a reference standard. The results were expressed as Trolox equivalent antioxidant capacity as mg (TEAC)/g extract.

2.10 Statistical analysis

All data were reported as mean ± standard deviation. The difference between each parameter was statistically analyzed by the Independent-samples t-test (IBM SPSS version 21.0). The level of significant was considered at $p < 0.05$.

3. Results and Discussion

3.1 Extraction yield and solubility of crude extract

The extraction yield by 70% ethanol solvent was 3.95%, demonstrating that the differences in solvents (70% and 95% EtOH) were statistically significant ($p < 0.05$), as shown in Table 1.

As previously examined by Hsiang-yu (2014), comparing the increasing the yields in varying extraction (stirring and reflux) in ethanol and aquas, the results showed that these extraction methods produced low yields when compared to a sonication extraction as mentioned by this author (Romee *et al*., 2022), using sonicated extraction in an aqueous medium (50% ethanol, 70% ethanol, 75% ethanol, water 100%) exhibited the highest yields.

3.2 Bioactive compounds

According to the result, TPC extraction using 95% ethanol provided the highest yield (408.83%).

The TFC and TPAC were slightly low in the 95% ethanol. The TPC and TFC contents were significantly different among the 95% ethanolic extract ($p < 0.05$), whereas TPAC was not vary significantly ($p > 0.05$).

According to Hsiang-yu (2014), the TPC in the ethanolic extract of *Z. officinale* was more effective than an aquas extraction due to the difference in solvents and extraction methods (stirring and reflux). Furthermore, Dai *et al*., (2010) verified that ethanolic extracts were another suitable solvent for polyphenol extraction that gave the best results. According to Romee *et al*., (2022), a sonication extraction with 95% ethanol was the most effective solvent for TPC extraction, whereas 70% ethanol was the most effective solvent for TFC extraction (Hu *et al*., 2011; Kumar *et al*., 2013). The results reported that a sonication extraction had the highest in TFC contents (Eberle *et al*., 2018).

Table 1 Extractable yield and bioactive compounds (Total phenolic - TPC, flavonoid - TFC, and proanthocyanidin - TPAC contents from *Z. officinale* extracts.

Z. officinale Extracts	% Yield	TPC (mg GAE/g extract)	TFC (mg QE/g extract)	TPAC (mg CE/g extract)
70% Ethanol	3.95±0.23% [a]	260.15±2.45 [b]	44.59±0.10 [a]	353.94±35.76 [a]
95% Ethanol	1.80±0.20% [b]	408.83±1.54 [a]	40.76±0.26 [b]	329.88±3.30 [a]

Mean ± S.D. ($n = 3$).
Different superscript letters (a,b) in the same column indicate significant differences values (The Independent-sample t-test, $p < 0.05$).

3.3 Antioxidant activities

The 95% EtOH extract exhibited the greatest antioxidant activity against the DPPH and FRAP radicals, whereas the 70% ethanol extracts reported the highest antioxidant activity according to ABTS. All the activities significantly different among the different solvents. ($p < 0.05$).

According to Hsiang-yu (2014), a literature review found that ethanolic extracts were more efficient than aqueous extracts in terms of bioactive components and antioxidant effects due to differences in techniques (stirring and reflux) and solvents. Eberle *et al*. (2018) reported the optimization of extraction methods by using different techniques (sonication, maceration, and soxhlet) and solvents to determine the effectiveness of bioactive compounds. This literature review reported that ethanol extraction by sonication was the most effective due to the shortest time and being the least expensive.

Table 2 Antioxidant activity (DPPH, FRAP and ABTS assays) from *Z. officinale* extracts.

Z. officinale Extracts	DPPH (mg TEAC/g extract)	FRAP (mg TEAC/g extract)	ABTS (mg TEAC/g extract)
70% Ethanol	844.36±1.11 [b]	1388.11±65.51 [b]	650.14±1.86 [a]
95% Ethanol	902.04±0.70 [a]	1893.13±5.49 [a]	616.55±10.02 [b]

Mean ± S.D. ($n = 3$).
Different superscript letters (a,b) in the same column indicate significant differences values (The Independent-sample t-test, $p < 0.05$).

4. Conclusion

Zingiber officinale can be extracted by sonication method with ethanolic solvents. It exhibited high bioactive compound and antioxidant activity especially 95% ethanolic extract. Results confirmed that zinger is one of important source for active ingredient for antioxidant or antiaging cosmetic.

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Utilization of Corn (*Zea mays*) Agro-residues as Natural Active for Cosmetic Application

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Abstract

Smog crisis and tiny particle matters (e.g., PM 2.5) from burning of agro-residues (e.g., corn) are recurring health problem every year in Northern Thailand. Corn (*Zea mays*) agro-residues are consisted of its stalk, (~ 48% of the total dry mass), leaves (28%), tassel (~1%), husks (8%) and cob (15%). This study aimed to utilize corn agro-residues (stalk, leaves, and tassel) (CARs) as natural active ingredient for cosmetic application. CARs were separated, cleaned, and extracted by five solvents [deionized (DI) water, 80% methanol, 80% ethanol, ethyl acetate and hexanes] with shaking method at 200 rpm for 24 h. Total phenolic (TPC) and flavonoid contents (TFC), and antioxidant activities (DPPH, ABTS and FRAP assays) as well as anti-tyrosinase activity for cosmetic application were determined. The results showed that the ethanol extract of corn leaves had the highest yield (14.6% dry basis). The highest TPC was statistically found in both ethanol and methanol extracts of tassel (791.9 ± 6.3 and 773.7 ± 17.2 mg GAE/g extract, $p<0.05$, respectively). While the highest TFC was statistically occurred in the hexanes extract of corn leaves (1,736.2 ± 166.3 mg QE/g extract, $p<0.05$). For antioxidant activity by DPPH and FRAP assays, the methanol extract of tassel had significantly highest antioxidant activities (756.8 ± 21.7 and 723.8 ± 7.7 mg TEAC/g extract, $p<0.05$, respectively). For ABTS assay, the highest antioxidant activity was significantly found in the ethanol extract of tassel (4,077.3 ± 28.1 mg TEAC/g extract, $p<0.05$). Moreover, the ethyl acetate of leaves extract showed the highest anti-tyrosinase (52% inhibition at 4 mg/ml, $p<0.05$). From these results suggested that corn agro-residues (especially its tassel and leaves) can be used as alternative source for natural active ingredient of cosmetic applications.

Keywords: Antioxidant activities; Anti-tyrosinase activity; Bioactive compound; Corn agro-residues; Utilization

1. Introduction

In Northern Thailand, smog crisis and tiny particle matters (e.g., PM 2.5) are recurring on February to April every year that affect to health problem such as respiratory disease, cardiovascular disease, skin disease, eye disease. One of the main causes is burning agricultural residues especially corn field. In 2010, corn cultivated area has totally 4.88 million rai with productivity of 4.6 million tons per year and crop residues of 6.8 million tons per year (Tengkaew and Wiwattanadate, 2014). Mostly, crop residues are eliminated by burning approximately 60% of total their residues. Besides, agro-residues can be reduced by produce as animal feed, fertilizer, or fuel briquette (Sinpiboon and Chaichana, 2021). Corn (*Zea mays*) known as a good source of phytochemicals such as phenolic acids, flavonoids, carotenoids, phytosterols and other phytochemicals providing pharmacological properties including hypoglycemic,

anti-inflammatory, antioxidant, and diuretic properties (Milind & Isha, 2013, Siyuan *et al.*, 2018). Corn agro-residues (CARs) are consisted of its stalk, (~ 48% of the total dry mass), leaves (28%), tassel (~1%), husks (8%) and cob (15%). Their corn residues might have some biological activities and can be developed as natural active in natural products such as cosmetic or related products to increased value and utilization.

Natural antioxidants are active ingredient that widely used in health care and cosmetic products due to their several beauty benefits such as anti-ageing, anti-wrinkle and skin lightening properties. Natural antioxidants can be found in phenolic compounds, flavonoid, carotenoid and vitamins in many plants including corn-residues. Previous study demonstrated that phenolic, lignan and flavonoid compounds were isolated from the methanol extract of cornstalk had anti-inflammatory, neuroprotective or hepatoprotective activity (Jung *et al*., 2014, 2015). An ethanol extract of corn leaves showed higher antioxidant than corn cob and corn husk extracts using DPPH assay (fidrianny *et al.*, 2016). Corn tassel, a male floral organ, also found that its ethanol extract had antioxidant activities using ABTS and DPPH assay and had antibacterial and anticholinergic properties (Al-khayri *et al*., 2022). However, comparison on bioactive and antioxidant activity between each CARs with different solvents extraction have little been investigated. Therefore, the aim of study was to evaluate corn agro-residues (stalk, leaves, and tassel) bioactive compounds including phenolic and flavonoid contents and cosmetic biological activity such as antioxidant and anti-tyrosinase activities from different corn agro-residues (stalks, leaves, and tassel). These CARs may be used as natural active ingredient in cosmetic application.

2. Materials and Methods

2.1 Chemicals and reagents

All chemicals and solvents were analytical grade. ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)), aluminum chloride ($AlCl_3$), catechin, dibasic phosphate, dimethylsulfoxide (DMSO), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 95% ethanol, ethyl acetate, ferric chloride, folin-ciocalteu, gallic acid, hexane, hydrochloric acid (37%), methanol, monobasic phosphate, potassium acetate (CH_3COOK), potassium persulphate ($K_2S_2O_8$), quercetin, sodium carbonate (Na_2CO_3), sulfuric acid, vanillin, 2,4,6-tris(2-pyridyl)-1,3,5-triazin (TPTZ), trolox, sodium metabisulfite.

2.2 Plant preparation

Corn (*Zea mays*) agro-residues (CARs) were procured from local farmer on July in Chiangrai province, Thailand. The samples were separated into stalks, leaves, and tassel of corn. Then these samples were cleaned and dried by hot air oven at 45°C until constant samples weight. Dried sample was milled as powder and kept in desiccator until used.

2.3 Extraction

CARs were extracted by five various solvents including deionized (DI) water, 80% methanol, 80% ethanol, ethyl acetate and hexanes with a ratio of sample: solvent (1:10 w/v). All extracts were shaken by using incubator shaker at 200 rpm for 24 hours. The mixtures were filtrated by Whatman filter paper No.1. Organic solvents (ethyl acetate and hexanes) were removed by rotary evaporator at 50°C. Extracts were freeze- dried and kept at -20°C.

2.4 Total phenolic content (TPC)

TPC was measured according to Thitipramote *et al.* (2016; 2022) with slightly modification. Briefly, 20 µL of CARs extracts was reacted with 100 µL of 0.2 M Folin-Ciocalteu reagent and 80 µL of 7.5% Na_2CO_3. The mixture was left to stand in the dark for 30 minutes at room temperature. The absorbance was measured at 765 nm using a microplate reader (Biotek, Epoch, USA). Gallic acid was used as a reference standard. The results were expressed as mg gallic acid equivalents (GAE)/ g extract.

2.5 Total flavonoid content (TFC)

TFC was measured according to Thitipramote *et al.* (2016) with slightly modification. Briefly, 25 µL of CARs extracts was mixed with 75 µL of ethanol, 140 µL of DI water, 5 µL of 10% (w/v) $AlCl_3$ and 5 µL of 1M CH_3 COOK, respectively. The mixture was incubated in the dark for 30 minutes at

room temperature. The absorbance was measured at 415 nm. Quercetin was used as a reference standard and the results were expressed as mg quercetin equivalents (QE)/g extract.

2.6 DPPH radical scavenging activity (DPPH)

The DPPH radical scavenging activity was performed as previously described (Thitipramote *et al.*, 2016). Briefly, 10 µL of CARs extracts was mixed with 190 µL of DPPH reagent. The reaction mixture was left to stand in the dark for 30 min at RT. The absorbance was measured at 515 nm. Trolox solution was used as reference standard. The percentage (%) of inhibition was calculated by following formula

$$\% \text{ inhibition} = \frac{(Abs \text{ control} - Abs \text{ extract})}{Abs \text{ control}} \times 100$$

The results were expressed as mg Trolox equivalent antioxidant capacity (TEAC)/ g extract).

2.7 ABTS radical scavenging activity (ABTS)

The ABTS radical scavenging activity was performed as previously described (Thitipramote *et al.*, 2016). ABTS solution was freshly prepared by mixing 7 mM ABTS with 2.45 mM $K_2S_2O_8$ (v/v). Then, the ABTS solution was diluted with 50 mM Phosphate buffer (pH 7) in a ratio of 1:20 (v/v). For analysis, 10 µL of CARs extracts was added into 190 µL of the diluted ABTS solution. The reaction was performed in the dark at room temperature for 15 minutes. The absorbance was measured at 734 nm. Trolox solution was used as reference standard. The percentage (%) of inhibition was calculated as mentioned above. The results were expressed as mg Trolox equivalent antioxidant capacity (TEAC)/ g extract.

2.8 Ferric reducing antioxidant power (FRAP)

Reducing power was measured as previously described (Thitipramote *et al.*, 2016). Briefly, FRAP solution was freshly prepared by mixing 10 mL of TPTZ solution in 40 mM hydrochloric acid with 10 mL of 20 mM ferric chloride and 100 mL of 0.3 M acetate buffer (pH3.6). For analysis, 30 µL of CARs extracts were mixed with 570 mL of FRAP solution. The mixture was kept in the dark for 15 minutes at room temperature and then the absorbance was measured at 593 nm using microplate reader. Trolox was used as a reference standard, and the results were expressed as mg Trolox equivalent antioxidant capacity (TEAC)/ g extract.

2.9 Anti-tyrosinase activity

Tyrosinase inhibition assays was performed as previously described (Wang *et al*., 2020). Briefly, L-DOPA were performed as substrate. The reaction mixture (1000 µL) contained 685 µL of phosphate buffer (0.05 M, pH 6.5), 15 µL of mushroom tyrosinase (2500 U/ mL), 200 µL of CARs extracts and 100 µL of 5 mM L-DOPA. After the addition of L-DOPA, the reaction was measured at 492 nm using microplate reader. Kojic acid was used as a positive control.

2.10 Statistical analysis

All data measurements were expressed as mean ± standard deviation. Data were compared and analyzed by one-way analysis of variance (ANOVA) test with Duncan's multiple range test (IBM SPSS version 21.0). A significant difference is considered at the level of $p < 0.05$.

3. Results and Discussion

3.1 Percentage yield of the CARs extract

Yield of extract were calculated as dry basis (%yield). The extraction yields of each part of corn with different solvents were shown in Table 1. Comparing solvent extraction from corn leaves and corn tassel, the ethanol extracts were exhibited higher extraction yield (14.62 and 10.25% yield, respectively) than other solvents at same sample. However, in corn stalks, the methanol extract showed higher yield (6.98 %yield) than those of other solvents.

3.2 Determination of total phenolic contents

Total phenolic contents (TPC) showed a significant difference between CARs extracts from five different solvents ($p<0.05$) as shown in Table 1. Phenolic compounds, a second metabolite of plants

including lignans, tannins, phenolics acids, stilbenes, and flavonoids, are mostly found in many plants. Phenolic compounds are responsible for the bitterness or account for the differences in the flavor and color of many fruits. The results showed that the methanol and ethanol extracts of CARs extract showed higher TPC than other solvents when comparing in the same part of CARs. The statistically highest TPC was found in both the methanol and ethanol of corn tassel extracts (791.9±6.3 and 773.7±17.2 mg GAE/g extract, $p<0.05$). Organic solvents extract more phenolic compounds than aqueous extract (Tok *et al*. 2017). Likewise, previous study showed that the ethanol was the best solvent for extracting phenolic compounds (0.1575% TPC) followed by methanol extract of corn tassel (Mohsen & Ammar, 2009).

3.3 Determination of total flavonoid contents

Total flavonoid content (TFC) of CARs extracts showed a significant different between CARs from five different solvents ($p<0.05$) as shown in Table 1. Low polarity of solvent exhibited higher TFC than high polarity of solvent at same sample. The statistically highest TFC was found in the hexane extract of corn leaves (1,736.2±166.3 mg QE/g extract, $p<0.05$). However, the lowest TFC was occurred in all DI extracts of these corn stalks, leaves, and tassel. Consistency, previous study showed that the DI water extract of cornhusk, corncob, and corn silk had the lowest TFC (Dong *et al.*, 2014).

Table 1 Extractable yield and bioactive compounds (total phenolic*:* TPC*)* and flavonoid*:* TFC contents*)* of CARs extracts including stalks, leaves, and tassel with five different solvent extractions.

CARs	Solvents	Yield of extracts (% dry basis)	Bioactive compound	
			TPC* (mg GAE/g extract)	TFC* (mg QE/g extract)
Stalks	DI water	1.74	422.2 ± 27.8^{f}	15.7 ± 5.2^{j}
	80% Methanol	6.98	588.9 ± 9.6^{c}	335.3 ± 6.6^{h}
	80% Ethanol	4.44	602.4 ± 9.5^{c}	$479.0 \pm 4.3^{f,g}$
	Ethyl Acetate	0.40	454.8 ± 10.4^{e}	655.0 ± 1.4^{c}
	Hexanes	0.62	467.5 ± 14.3^{e}	915.8 ± 3.8^{b}
Leave	DI water	7.43	550.0 ± 26.1^{d}	15.1 ± 0.0^{j}
	80% Methanol	13.25	736.5 ± 25.8^{b}	423.3 ± 31.7^{g}
	80% Ethanol	14.62	551.6 ± 5.5^{d}	$455.3 \pm 11.5^{f,g}$
	Ethyl Acetate	3.84	267.5 ± 33.3^{h}	$520.4 \pm 11.5^{e,f}$
	Hexanes	5.89	$575.2 \pm 20.8^{c,d}$	$1{,}736.2 \pm 166.3^{a}$
Tassel	DI water	6.55	734.4 ± 19.5^{b}	54.0 ± 5.0^{j}
	80% Methanol	7.63	791.9 ± 6.3^{a}	249.2 ± 2.9^{i}
	80% Ethanol	10.25	773.7 ± 17.2^{a}	$570.83 \pm 3.8^{d,e}$
	Ethyl Acetate	4.60	$401.4 \pm 8.6^{f,g}$	876.3 ± 2.5^{b}
	Hexanes	6.56	387.9 ± 7.7^{g}	$600.8 \pm 11.8^{c,d}$

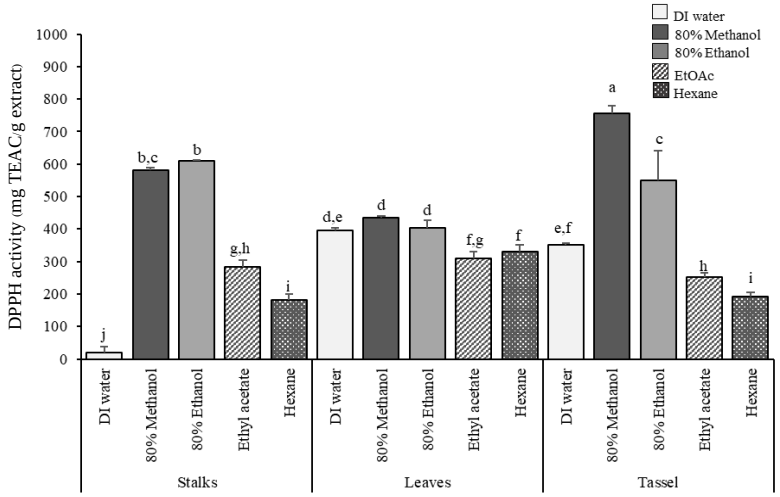
*Values are given as mean ± S.D. (n=3)
*Different letters in the same column indicate significant differences values ($p<0.05$) (ANOVA, Duncan's test).

3.3 Antioxidant activity of the CARs extract

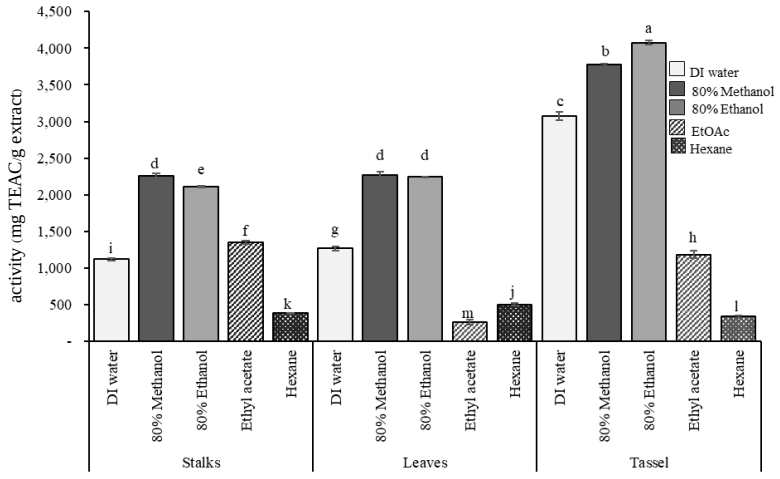
Antioxidant activities were performed by using DPPH, ABTS and FRAP assays. DPPH assay was based on the ability of a compound that can donate a hydrogen atom from 2,2'-diphenyl-1-picrylhydrazyl converted into 2,2'-diphenyl-1-picrylhydrazine. (Pyrzynska and Pękal, 2013). DPPH radical scavenging activity of CARs extracts were a significant different between CARs extracts as shown in Figure 1A. Comparing between solvents, both methanol and ethanol extracts showed higher antioxidant activity than other solvents. The methanol extract of corn tassel exhibited the statistically highest DPPH radical scavenging activity (756.8±21.7 mg TEAC/g extract, $p<0.05$).

ABTS assay is tested on the capability of hydrogen donor of antioxidant, from free radical (2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) ammonium salt radical cation or $ABTS^{\bullet+}$) to ABTS (Sharopov *et al*., 2015). The tendency of ABTS activity was similar to DPPH radical scavenging activity as shown in Figure 1B. Both methanol and ethanol showed higher antioxidant activity than other

(A)



(B)



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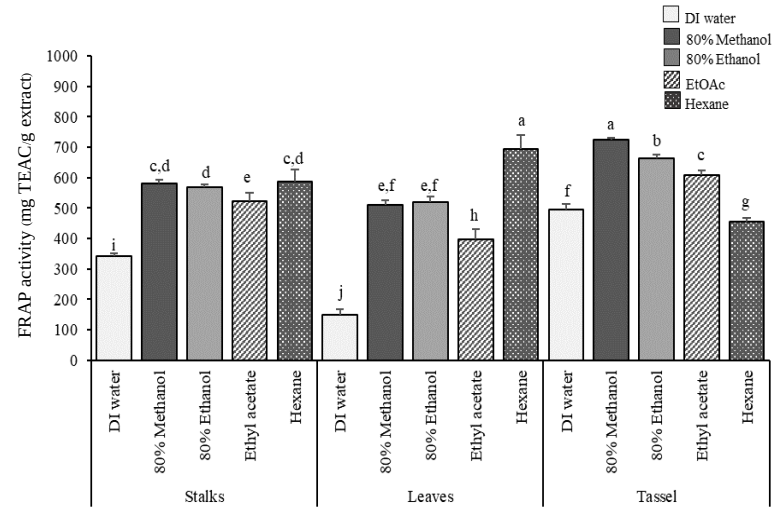

Figure 1 Antioxidant activities by using (A) DPPH radical scavenging activity (B) ABTS radical scavenging activity and (C) Ferric reducing antioxidant power (FRAP) of CARs including corn stalks, leaves, and tassel with five various solvents (DI water, 80% methanol, 80% ethanol, ethyl acetate and hexanes. The error bars represent the standard deviation of mean. The different letters indicate significant difference values ($p< 0.05$).

solvents. The greater ABTS antioxidant activity was found in the ethanol extract of corn tassel (4077.31±28.12 mg TEAC/ g extract, $p<0.05$) followed by the methanol and DI water extract of corn tassel ($p<0.05$; Figure 1B).

FRAP assay is different from DPPH and ABTS assay due to it does not involve with free radicals. This method is used to examine the antioxidant competency by the redox reaction of ferric ion (Fe^{3+}) to ferrous (Fe^{2+}) (Sharopov *et al.*, 2015). FRAP activity results were shown in Figure 1C. The results were indicated that the methanol extract of corn tassel and the hexane extract of corn leaves had significant the highest antioxidant activity (723.8±7.7 and 694.0±45.6 mg TEAC/g extract, $p<0.05$). Comparing each CARs extract in same solvent extraction, the results showed that corn tassel had higher antioxidant activity using FRAP assay than other CARs including corn stalks and corn leaves, respectively (Table 1C).

Similar to previous study, the methanol extract of corn tassel had antioxidant capacity using DPPH and ABTS assay (Al-khayri *et al.*, 2022). Other part of CARs, the 80% ethanol and the 80% methanol were also efficient in extracting antioxidant constituents in cornhusk, corncob, and cornsilk (Dong *et al.*, 2014).

3.4 Anti-tyrosinase activity of the CARs extract

Tyrosinase as the rate-limiting enzyme for biosynthesis of melanin, catalyzes the oxidation the substances that transformed to melanin. Therefore, tyrosinase inhibitors gained a lot of use in cosmeceutical for use as whitening agents. The results of anti-tyrosinase activity from CARs extracts were shown in Figure 2. In each CARs, either ethyl acetate or DI water extractions tended to be higher inhibition of tyrosinase activity than other solvents at same sample. The ethyl acetate extract of corn leaves had greatest significant inhibitory tyrosinase activity at concentration 4 mg/ml (52.6±5.4 %inhibition, $p<0.05$) and ranging in the order: DI water extract of corn leaves > DI water extract of corn tassel as indicated in Figure 2. However, the inhibitory activity was undetectable in the hexane extracts of corn stalks and tassel and the ethyl acetate extract of corn tassel. Previous study showed that waxy corn silk had anti-tyrosinase activity with an IC_{50} value at 3082.28±347.98 μg/ml (Hongsuwan & Satthanat, 2018). Wang *et al.*, (2020) demonstrated that ethyl acetate soluble fraction from stream exploded of corn stalks had anti-tyrosinase activity with an IC_{50} of 0.29 mg/ml.

4. Conclusion

Corn agro-residues including their stalks, leaves, and tassel composed of bioactive compounds (phenolic and flavonoid contents) and had bioactivities for cosmetic (antioxidant and anti-tyrosinase activities). Total phenolic content and antioxidant capacities tended to be greater in CARs tassel extract

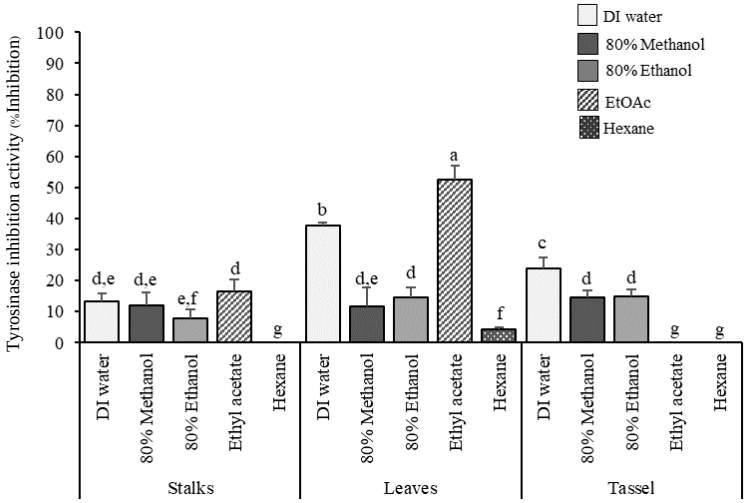

Figure 2 Anti-tyrosinase inhibitory activity (%) of CARs including corn stalks, leaves, and tassel at concentration 4 mg/ml with five various solvents (DI water, 80% methanol, 80% ethanol, ethyl acetate and hexane. The error bars represent the standard deviation of mean. The different letters indicate significant difference values ($p< 0.05$).

(especially methanolic and ethanolic extraction). Furthermore, the tendency of total flavonoid content and tyrosinase inhibitory activity was high in these CARs leaves extracts (especially lower polarity solvents ethyl acetate and hexanes extractions). Therefore, corn agro-residues (especially its tassel and leaves) can be value-added and utilization for alternative source for natural active ingredient in cosmetic and related applications.

5. Acknowledgements

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Development of oral rinse containing *Terminalia chebula* Retz fruit extract as an antibacterial agent against *Streptococcus mutans*

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Abstract

Oral rinse is an antiseptic solution used for enhancing oral hygiene that could be a part of the preventive dentistry regimen at the present time. *Streptococcus mutans* is considered to be a major pathogen of dental caries due to its ability to adhere to the tooth surface by producing extracellular polysaccharide. This can lead to biofilm formation and the ability to produce organic acid which attacking on the tooth enamel. The objective of this research was to develop an oral rinse containing *Terminalia chebula* Retz fruit extract with antibacterial activity against *S. mutans*. The *T. chebula* fruit extract was prepared by maceration using 70% (v/v) ethanol solution. The extracts were filtered and the solvent was removed by rotary evaporator. Antibacterial activity against *S. mutans* was performed by agar well diffusion assay. The minimal inhibitory concentration and the minimal bactericidal concentration of an extract were investigated by broth dilution assay. The results revealed that the *T. chbula* fruit extract possessed high antibacterial activity against *S mutans*. The minimal inhibitory concentration and the minimal bactericidal concentration of an extract were 1.56 mg/ml and 6.25 mg/ml, respectively. The oral rinse formulation containing *T. chebula* fruit extract was developed. The organoleptic and physicochemical stabilities of the formulation were investigated under accelerated conditions. The results showed that the developed oral rinse had good organoleptic and physicochemical stabilities. In conclusion, the formulation of an oral rinse containing *T. chebula* Retz fruit was stable at the end of the accelerated testing. Therefore, it can be used as a natural antibacterial active in oral rinse formulation that could be further studied in clinical trials.

Keywords: Antibacterial; Dental caries; Oral rinse; *Streptococcus mutans*; *Terminalia chebula* Retz

1. Introduction

Dental caries is a common chronic disease which affects both oral health and the quality of life. *Streptococcus mutans* is considered to be a major pathogen associated with the initiation of dental caries due to its ability to adhere to the tooth surface by producing extracellular polysaccharide which can leads to biofilm formation. Furthermore, this bacteria is highly acidogenic which can cause an acidic environment that attacking on the tooth enamel (Abranches *et al.*, 2018; Forssten *et al.*, 2010; Tanzer *et al.*, 2001). Practicing a good oral hygiene routine is the best way to prevent dental caries and periodontal disease. Oral rinses are an antiseptic solution used to rinse the mouth in order to enhance oral hygiene and are considered as one of the most effective and safe delivery system to decrease oral microbes (Sadeghi *et al.*, 2011; Hambire *et al.*, 2015). Chlorhexidine is the most effective chemical

antiplaque agent that has been widely used in antiseptic oral rinse, however, it may cause side effects in long usage including tooth staining, sore mouth and/or throat, tongue irritation and wheezing/shortness of breath (Brookes *et al.*, 2020; Sharma *et al.*, 2014). Therefore, herbal oral rinse containing natural substances such as plant extracts which have minimum side effects and effective have been attracting much attention as an alternative substance in oral caring (Dabholkar *et al.*, 2016; Rekha *et al.*, 2014).

Terminalia chebula exhibited a number of medicinal activities has been extensively used as a folk medicine from ancient times (Bag *et al.*, 2013). *T. chebula* fruit has high contents of phenolic compounds including phenolic, tannin and flavonoid (Nigam *et al.*, 2020). Several studies reported that *T. chebula* fruit extract possessed antimicrobial activity against *S. mutans* and can be considered as a anti-cariogenic agent (Aneja & Joshi, 2009; Jagadish *et al.*, 2009; Nayak *et al.*, 2014). However, the development of oral product containing *T. chebula* for antibacterial has little been studies. Therefore, the objective of this research was to develop an oral rinse containing *Terminalia chebula* fruit extract with antibacterial activity against *S. mutans*.

2. Materials and Methods

2.1 Plant extraction

T. chebula fruit were collected in November 2018 from Loei Province, Thailand. The plant extraction was prepared according to Beg et al. (2012). Briefly, 25 g of the dried pericarp powder of *T. chebula* was macerated with 150 ml of 70% ethanol with shaking at 150 rpm for 24 hours at room temperature. After that the extract solution was filtered through a Whatman No.1 filter paper and organic solvent was removed using a vacuum rotary evaporator. The crude extract was then stored in a tight container until further use. The yield of extract was calculated using the following equation.

Yield (%) = (Weight of *T. chebula* crude extract)/ Weight of *T. chebula* pericarp powder) x 100

2.2 Chemical composition analysis

The chemical composition of *T. chebula* crude extract was determined using high performance liquid chromatography (HPLC). Gallic acid (GA) and ellagic acid (EA) were used as a reference standard. The separation was done in a VerticepTM GES C8 column (4.6 x 250 mm, 5µ particle size), mobile phases consisted of (A) 0.05% trifluoroacetic acid in water, and (B) 0.05% trifluoroacetic acid in acetonitrile using a gradient elution and the flow rate was set at 1.0 mL/min with the controlled temperature at 40º C. Photo diode array 190-400 nm detector was set at the wavelength of 259 nm and injection volume was 10 µL.

2.3 Antibacterial activity of *T. chebula* extract

Antibacterial activity of *T. chebula* extract against *S. mutans* was determined by agar well diffusion method according to Ahmed & Beg (2001). The broth culture of a mid log phase of *S. mutans* ATCC 25175 (10^7 cfu/ml) was prepared using a brain heart infusion broth (BHI) supplemented with 2 % glucose at 37 °C with shaking at 200 rpm. Then, the bacteria suspension was inoculated on BHI agar plate using sterile cotton swab. After that, a hole with a diameter of 6 mm was punched with a sterile cork borer and a 40 µl of a different concentration of *T. chebula* extract solution was introduced into the well. The agar plate was then incubated at 37 °C for 18 hours. 0.5% chlorhexidine and BHI broth were used as a positive control and negative control, respectively. Antimicrobial activity was determined by measuring inhibition zone diameters. The experiment was performed in triplicate.

2.4 MIC and MBC of *T. chebula* extract

Minimal inhibitory concentration (MIC) of *T. chebula* extract against *S. mutans* was measured by broth macrodilution method. In brief, the extract solution was diluted two-fold serially with brain heart infusion broth (BHI) ranging from 0.78-25 mg/ml. and then 1 mL of each concentration of extract solution were given in each test tube containing 9 mL of BHI broth. Later, 1 mL of working inoculum suspension ($5x10^5$ CFU/mL) was added to each tube. The negative control consisted of BHI broth and inoculum, and the blank control consisted only the BHI broth. The sample was then incubated for 24h at 37ºC. The MIC was defined as the lowest concentration showing 80% growth inhibition compared

to the growth control tube. The minimal bactericidal concentration (MBC) of an extract was performed by placing each sample dilution on BHA. The experiments were performed in triplicate.

2.5 Oral rinse formulation

Oral rinse formulation containing *T. chebula* extract was prepared by adding 0.5% w/w in formula of *T. chebula* extract according to cytotoxicity concentration of *T. chebula* extract in fibroblast cell reported (Prompamorn *et al.,* 2022). The formulation of oral rinse shown in Table 1

Table 1 Formulation of oral rinse containing *T. chebula* extract.

Ingredients	Function	%w/w in formula
DI water	Diluent	qs to 100
Propylene glycol	Solubilizer	10
glycerin	Humectant	5
Polysorbate 20	Surfactant	2
T. chebula extract	Active ingredient	0.5
Sodium chloride	Flavoring agent	0.2
Sodium saccharin	Sweetener	0.1
Sodium benzoate	Preservative	0.1
Triethanolamine	pH adjust	0.15
Menthol	Cooling agent	0.05

2.6 Organoleptic testing

The physical appearance of prepared oral rinse formulation was evaluated including color, pH, viscosity and homogeneity. The color of oral rinse formulation was determined by Chroma meter CR-400. The Viscosity of the formulation was determined by Brookfield viscometer DV-I Prime at 60 rpm, using spindle no 62. The pH of the formulation was measured by pH meter and the homogeneity of the formulation was determined by visual appearance.

2.7 Accelerated stability testing

Stability test was conducted under heating cooling condition. In brief, the oral rinse formulation was kept at 4.0±0.1°C in refrigerator for 48 hours and 45±0.1°C with 75% relative humidity for 48 hours. This process continued for 24 days. The physicochemical properties of the formulation including color, pH and the amount of gallic acid and ellagic acid were monitored at the beginning and the end of the stability tested.

3. Results and Discussion

3.1 Plant extraction

The prepared *T. chebula* fruit crude extract has dark-brown color (Figure.1) The yield of an extract was 53.75% (wet basis) which was higher than previously study conducted by Nayak *et al.* (2014) (48%).

3.2 Chemical composition analysis

The HPLC analysis showed that *T. chebula* extract possessed the contents of gallic acid and ellagic acid and ellagic acid was the major constituent of an extract. From the chromatogram, the content of ellagic acid and gallic acid in the extract was 36.671 mg/g crude extract and 14.542 mg/g crude extract, respectively. The chromatograms of gallic acid standard compound, ellagic acid standard compound and *T. chebula* extract were shown in Figure 2. The result of an experiment was in agreement with Lee *et al.,* 2017b; Saha & Verma (2018) and Pfundstein *et al.* (2010). However, the content of ellagic acid in an extract was higher than previously study conducted by Pfundstein *et al.* (2010) due to the different extraction method.

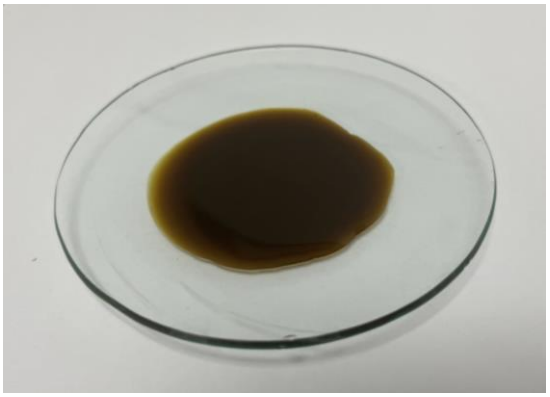

Figure 1 *T. chebula* extract

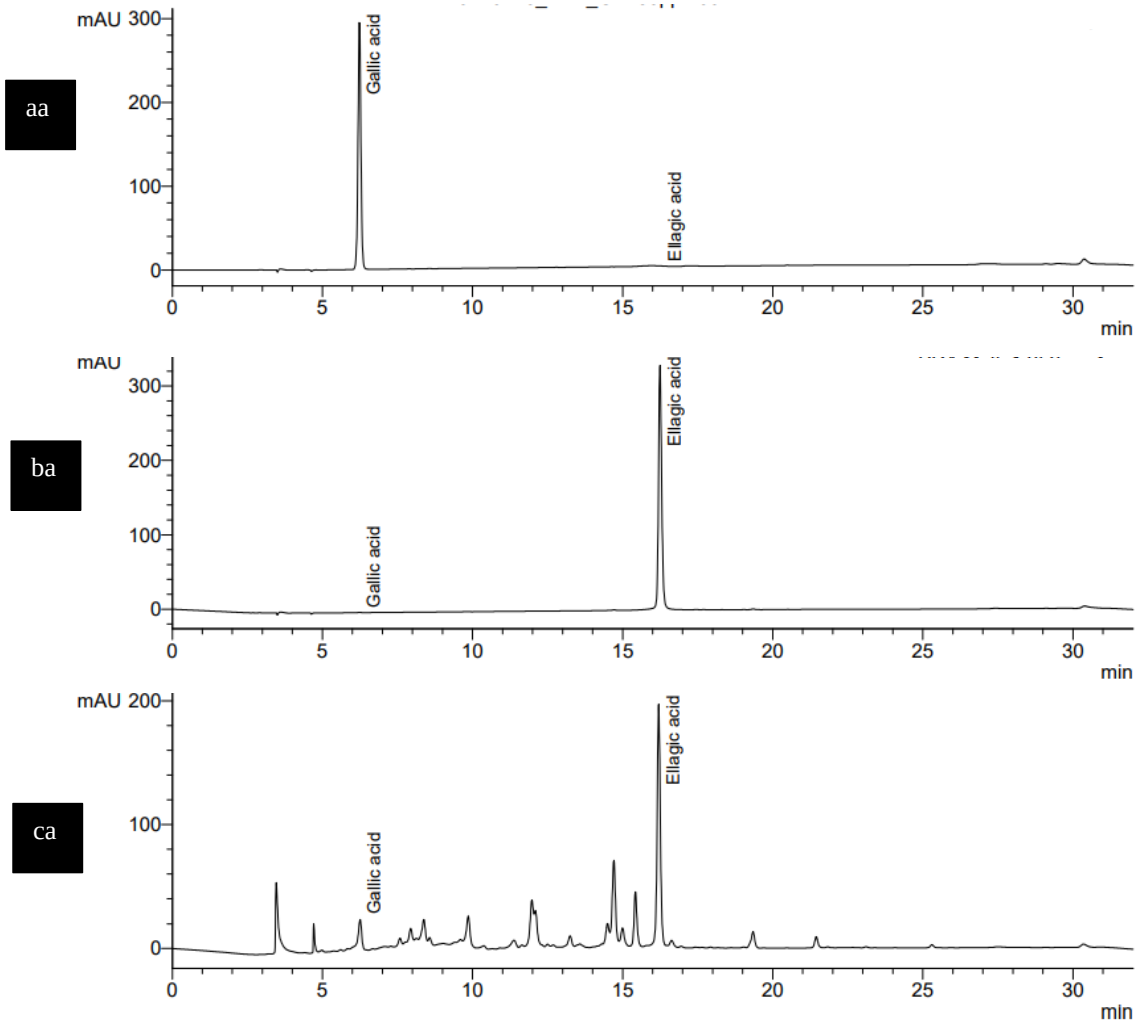

Figure 2 HPLC chromatogram of (a) gallic acid standard, (b) ellagic acid standard and (c) *T. chebula* extract

3.3 Antibacterial activity of *T. chebula* extract

The antibacterial activity of *T. chebula* extract according to agar well diffusion assay showed that the extract possessed strong inhibitory activity against *S. mutans* bacteria and the degree of inhibition increased with the concentration of the extract. The antibacterial activity of the extracts was shown in Table 2. The MIC and MBC of an extract against *S. mutans* performed by broth macrodilution were

1.56 mg/ml and 6.25 mg/ml, respectively. The results were in agreement with previous studies (Lee *et al.,* 2017; Rekha *et al.,* 2014) which revealed that *T. chebula* extract exhibit anticaries activity. The antibacterial activity of the extract probably due to the ellagic acid and gallic acid which found in the extract. The gallic acid effected the membrane properties of bacteria (charge, intra and extracellular permeability, and physicochemical properties) which leads topore formation in the cell membrane (Borges *et al.,* 2013) while ellagic inactivated microbial adhesins, enzymes, cell envelope transport proteins, and complex with polysaccharide (Loo *et al.,* 2010).

3.4 Organoleptic testing

The physical appearance of prepared oral rinse formulation was showed in Figure 4. The formulation was homogeneity. The color of oral rinse formulation was dark brown. The color values that obtained from the colorimeter in term of L*, a*, and b* were 14.10±0.61, 0.66±0.02 and 1.21±0.15, respectively. The pH was 6.31which fulfilled the acceptance value of Thai community product standard of herbal breath freshener. The viscosity was 3.5 cp. at 60 rpm at room temperature.

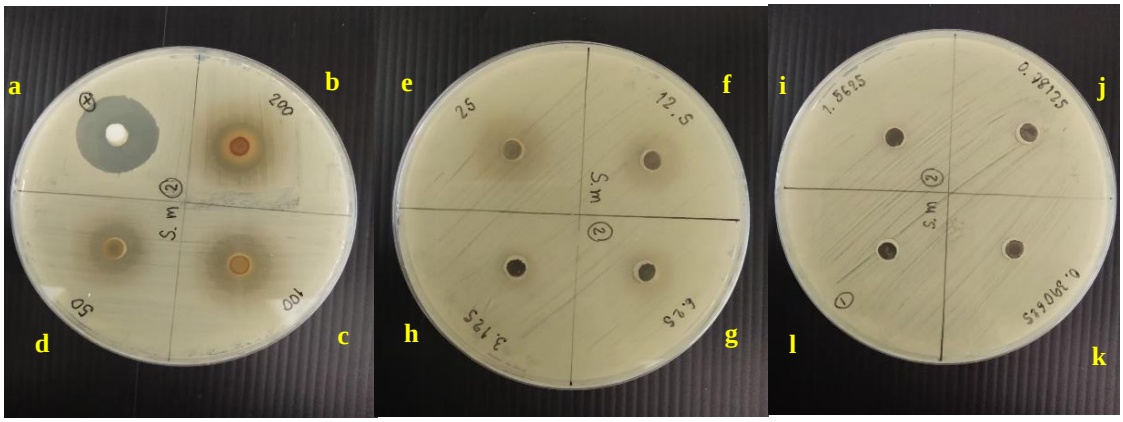

Figure 3 Zone of inhibition of *S. mutans* tested with (a) negative control, (b) *T. chebula* extract at a concentration of 200 mg/mL, (c) 100 mg/mL, (d) 50 mg/mL, (e) 25 mg/mL, (f) 12.5 mg/mL, (g) 6.25 mg/mL, (h) 3.125 mg/mL, (i) 1.5625 mg/mL, (j) 0.78125 mg/mL, (k) 0.390625 and (l) negative control.

Table 2 Antibacterial activity of *T. chebula* extract at various concentration (0.3-200 mg/mL) against *S. mutans* by agar well diffusion assay.

T. chebula extract (mg/mL)	Zone of inhibition (mm)
200	13.53±0.3[a]
100	12.46±0.1[b]
50	7.33±0.2[c]
12.5	ND
6.25	ND
3.125	ND
1.5625	ND
0.78125	ND
0.390625	ND
0.5% Chlorhexidine (Control)	16.36±0.2[d]

Remark: Values are given as mean ± S.D. from triplicate, The different superscript letter (a, b, c, d) in the same column represents significant difference when compared with each concentration in variables at $p<0.05$ (one way ANOVA with Tukey test). ND = not determined.

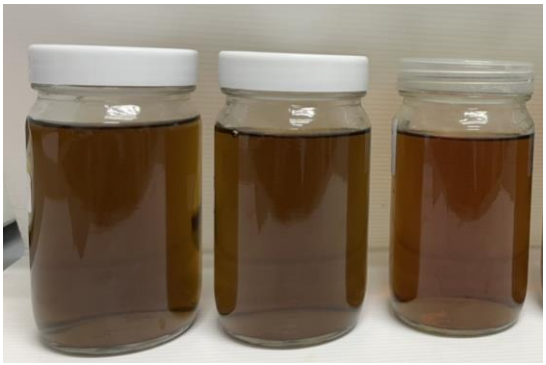

Figure 4 The physical appearance of oral rinse composed of *T. chebula*

3.5 Accelerated stability testing

The result of physicochemical properties of the oral rinse composed of *T. chebula* extract showed that the formulation including color, pH and the amount of gallic acid and ellagic acid of initial condition and after the stability tested are shown in Table 3.

According to the accelerated stability testing of the oral rinse formulation, the result showed that the formulation of an oral rinse containing *T. chebula* Retz fruit was stable at the end of the accelerated testing. Interestingly, the content of gallic acid in the oral rinse formulation was higher than ellagic acid while ellagic acid was the major constituent of *T. chebula* extract. This result are in agreement with Panichayupakaranant *et al.* (2010) which suggested that the hydrolysis reaction of the ellagic acid was occurred in the aqueous solution and the instability of this compound in the aqueous solution should be considered. The remaining percentage of gallic acid and ellagic acid in the oral rinse formulation after 24 days of accelerated test were higher than 90% (gallic acid 93.02%, ellagic acid 99.19%). Based on the extract's stability, *T. chebula* extract could be recommended to be used as an active ingredient in oral rinse product.

Table 3 Physicochemical properties of the oral rinse compose of *T. chebula* extract during initial and after stability test.

Parameter	Initial condition	After stability test
Color	L* 14.10±0.61	L* 13.47±0.36
	a* 0.66±0.02	a* 0.41±0.03
	b*1.21±0.15	b*1.04±0.12
pH	6.31	5.89
viscosity	3.5 cp.	15 cp.
Gallic acid	65.182 mg/L	60.635 mg/L
Ellagic acid	39.528 mg/L	39.208 mg/L

4. Conclusion

In this study, the chemical composition of *T. chebula* extract and the antibacterial activity against *S. mutans* were analyzed as well as the organoleptic and accelerated stability test of the formulation of oral rinse containing *T. chebula* extract. The result from the experiment suggested that *T. chebula* extract possessed strong antibacterial activity against *S. mutans*. The oral rinse formulation containing *T. chebula* extract was stable at the end of the accelerated test and the percentage of gallic acid and ellagic acid, the major constituent of an extract in the oral rinse formulation after accelerated test were higher than 90%. Therefore, *T. chebula* extract can be used as a natural antibacterial active in oral rinse formulation. However, the clinical trials of the antibacterial activity of the oral rinse formulation needs further study and evaluation.

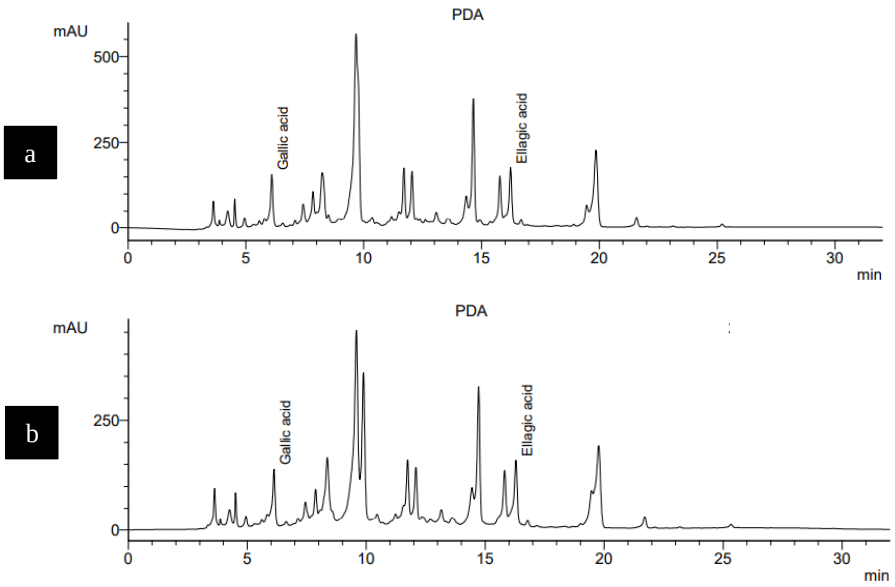

Figure 4 The amount of gallic acid and ellagic acid in the oral rinse compose of *T. chebula* extract at (a) initial condition and (b) after the stability tested

5. Acknowledgements

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Skin lightening effect of star grass (*Hypoxis aurea* Lour.) extract

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Abstract

Consumer interest in cosmetic skin care products made with natural ingredients has increased recently. Particularly, lightening items that help lessen melanin synthesis and prevent abnormal skin pigments, freckles, melasma, and dark spots were the most well-liked. The purpose of this study was to investigate the extraction solvent for star grass rhizomes and determine its ability to lighten skin. In this study, deionized water, 50% (w/v) and 95% (w/v) ethanol were utilized as the extraction solvents. The highest total phenolic content (0.20±0.02 mg GAE/mL) was found in water extract, while DPPH radical scavenging (IC_{50} of 0.58±0.00 mg/mL), FRAP (0.18±0.01 mg AAE/mL) and tyrosinase inhibition (IC_{50} of 0.68±0.01 mg/mL) was found in 50% ethanolic extract. This 50% ethanolic extract was then evaluated for cytotoxicity and the extract (26 mg/mL) showed no toxicity with 109.39±4.6% of cell viability. The base emulsion cream was produced, and 3% extract was added. On 32 participants, the product's potential for irritating skin was examined and the results revealed no irritation. Then, 28 volunteers participate in the further clinical test to determine the changing of melanin content after using the cream for 8 weeks. After using the product for 6 and 8 weeks, the amount of melanin decreased by 6.68±0.30% and 6.87±0.28%, respectively. The findings showed that the amount of melanin changed after 6 and 8 weeks was significantly lower than baseline (p<0.05). However, there was no discernible reduction in the cream base. Additionally, the products increased skin hydration from the baseline consistently by 6.68±0.35% and 32.70±0.26% throughout the course of 6 and 8 weeks. They demonstrated a significant difference after 6 to 8 weeks of application (p<0.05), respectively. According to the findings, star grass extract can be employed as an active component in cosmetic products to decrease melanin content and promote skin moisture.

Keywords: Star Grass, Melanin, Brightening, Redness, Moisturizing

1. Introduction

The major source of skin color in humans is melanin (pigment-producing cells) produced in highly specialized cells known as melanocytes, located in the outermost layer of the epidermis. Melanocytes are dendritic cells in close contact with neighboring keratinocytes. Together, they form the epidermal melanin unit, which creates and distributes melanin in the skin, hair, and eye colors. There are two types of melanin composed of eumelanin (black or brown) and pheomelanin (red or yellow) that exhibit distinct physical and biological properties (Busca & Ballotti, 2000; Yoon *et al*., 2003; Lin & Fisher, 2007; Chang, 2012).

Tyrosinase, a copper-containing enzyme, is a key enzyme for melanin biosynthesis in plants, microorganisms, and mammalian cells. In addition, tyrosinase enzyme inhibitors have been investigated in cosmetics and pharmaceuticals to prevent the overproduction of melanin in basal epidermal layers,

termed melanogenesis, which is widely used as a depigmentation agent (Sánchez-Ferrer *et al*., 1995; Parvez *et al*., 2007). In recent years, a consumer interested in cosmetic skin care products made with natural ingredients. Mainly, brightening items that reduce melanin synthesis which are the most popular because they could prevent abnormal skin pigmentation, freckles, melasma, and dark spots.

Melanin plays an important role in protecting human skin from the harmful effects of UV radiation from sunlight because humans naturally create melanin pigments for photo-protection. On the other hand, when skin exposure to UV radiation for a long time, melanogenesis is enhanced via the activation of tyrosinase, resulting in excessive production of melanin and occur adverse effects such as DNA-damaged epidermal hyperplasia, collagen breakdown, and inflammation (Gillbro & Olsson, 2011; d'Ischia *et al*., 2013). Therefore, skin-brightening ingredients with hypo-pigmentation abilities like anti-melanogenesis activity or refer to anti-melanogenesis agents are essential.

Star grass (*Hypoxis aurea* Lour.), Thai name: WanTan-Diao, is a plant from the genus Hypoxidaceae. It can be found in China, Japan, and Southeast Asia countries, including Thailand. The previous literature investigation reported the isolation of phenolic glycosides such as aureaside A and aureaside B were only chlorinated aromatic glycoside which isolated from genus *Hypoxis* until present. Other isolation compounds were flavonoids and steroids. Furthermore, other compounds by ethanolic extract of star grass composed of quercetin-3-O-β-D-glucoside, kaemferol-3-O-β-D-glucoside, apigenin-5-O-β-D-glucopyranoside, α-spinasterol, 2,6-dimethoxy-benzoic acid, 1H-indole-3-carboxylic acid, (2S, 3R, 4E, 8E)-1-(β-D-ghicopyranosyloxy)-3-hydroxy-2 [((R)-2'-hydroxyeicosanoly)amino]-9-methy-4,8-octadeeadiene, n-dotriacontanol, 14,15-eicosenicacid, lignoceric acid, β-sitosterol, and daucosterol (Cheng *et al*., 2009; Zhong-Quan *et al*., 2011). In traditional Thai medicine, tubers of star grass have been used for maintaining blood circulation and were later externally used for cosmetic and cosmeceutical purposes. Additionally, the star grass extracts from leave and tuber showed antioxidant activity, anti-tyrosinase, anti-melanogenesis, and collagen biosynthesis stimulating ability (Boonpisuttinant *et al*., 2013; Boonpisuttinant *et al*., 2014). However, there have been no reports of a clinical trial to assess the skin brightening efficacy of star grass extract. Thus, this research aims to investigate the phenolic content, antioxidant activities, cytotoxicity, skin irritation, and clinical study evaluation of star grass extract.

2. Materials and Methods

2.1 Materials

Ascorbic acid, dimethyl sulfoxide (DMSO), ferric chloride, Folin-ciocalteu reagent, gallic acid, kojic acid, MTT formazan, sodium bicarbonate, sodium hydroxide, trichloro acetic acid, tyrosinase from mushroom, 1,1-diphenyl 1-2-picrylhydrazyl, and 3,4-dihydroxy-L-phenylalanine were purchased from Sigma-Aldrich Co., St. Louis, MO, USA. Ethanol was purchased from Merck, Darmstadt, Germany. Ferric chloride and potassium ferricyanide were purchased from Fisher Scientific, Waltham, MA, USA. Dimethyl sulfoxide (DMSO) was purchased from Bio Basic Inc., Canada. Dulbecco's Modified Eagle's Medium was purchased from Caisson Labs, Utah, USA. Fetal bovine serum, penicillin streptomycin solution, phosphate buffered saline (PBS), and typsin/EDTA solution were purchased from Gibco, Grand Island, NY, USA. Butylene glycol, cetearyl alcohol, glycerin, glyceryl stearate, lexemul 561, Novemer EC-1 polymer, phenoxyethanol, and sodium lauryl sulfate were purchased from Namsiang, Bangkok, Thailand. $B_{16}F_{10}$ Melanoma cell (melanocyte cells) was received from The NIC company, Chiang Rai, Thailand.

2.2 Extraction

Rhizomes of star grass were harvested from Nam Kian, Phu Phiang, Nan, Thailand during November to December in 2020. The fresh rhizomes were cleaned and dried in an oven (model ED-S 56, Binder, Tuttlingen, Germany), which maintained temperature of at 45ºC for 120 hours. Then, the dry rhizomes were blended with a blender (HR2115/02, Philips, Bangkok, Thailand) to obtain small pieces. Dried rhizomes were extracted with three different solvents (deionized water, 50% and 95% alcohol) with a ratio 1:5 (w/v) and sonicated in a sonication bath (Elmasonic S 10 H, Gottlieb-Daimler, Germany) for 20 minutes at room temperature. All extracts were filtered using a Buchner funnel through filter paper (Whatman® no.1) to remove residue, then dried by using rotary evaporator (Buchi R-114 Rotary Vap System, BUCHI Corporation, New Castle, DE, USA) at 40°C and kept in a freezer until

analysis.

2.3 Determination of Total Phenolic Content

The total phenolic content (TPC) of extracts was determined using Folin-Ciocalteu assay (Vichit & Saewan, 2016). The extract (12.5 µL) was mixed with 50 µL of deionized water, 20 µL of Folin-Ciocalteu reagent and 125 µL of 7% sodium bicarbonate. The mixture was incubated for 90 minutes at room temperature. Then, the absorbance was read using a microplate reader (BMG LABTECH/SPECTRO star Nano, Ortenberg, Germany) at 750 nm. TPC was expressed as gallic acid equivalent (mg GAE/mL extract).

2.4 DPPH Radical Scavenging Activity

DPPH radicals scavenging activity of extracts was evaluated by the followed protocol of Vichit and Saewan (2015). 5 µL of extract and 0.1 mM of DPPH solution (195 µL) were mixed and left to react at room temperature for 30 minutes under dark conditions. The absorbance of the mixture was measured at 515 nm using a microplate reader. The antioxidant activity was calculated as follows:

$$\text{DPPH scavenging activity (\%)} = [(A_{control} - A_{sample})/A_{control}] \times 100 \quad (1)$$

Where $A_{control}$ is the absorbance of the control (without extract) and A_{sample} is the absorbance in the presence of the test sample. Then, the DPPH radical scavenging activity of the extract was expressed as 50% inhibition concentration (IC_{50}) and ascorbic acid was used as the positive standard.

2.5 Ferric Reducing Power Activity

The ferric reducing power (FRAP) assay was performed according to the method of Vichit and Saewan (2016). The extracts (25 µL) were mixed with 50 µL of 1% potassium ferricyanide and incubated for 60 minutes at room temperature. Then, 25 µL of Trichloro acetic acid and deionized water (75 µL) were added and the absorbance was measured at 700 nm using a microplate reader as absorbance 1 (A1). Next, 0.1% ferric chloride (25 µL) was added to the solution, and the mixture absorbance was again measured at 700 nm as absorbance 2 (A2). The optical density of sample was calculated using the following equation:

$$\text{Optical density} = (A2 - A1)_{sample} - (A2 - A1)_{control} \quad (2)$$

Where $A_{control}$ is the absorbance of the control (without extract) and A_{sample} is the absorbance in the presence of the test sample. The reducing power activity results were expressed as ascorbic acid equivalents (mg AAE/mL extract).

2.6 Anti-Tyrosinase Activity

A tyrosinase inhibitory activity assay by the dopachrome method, in which L-DOPA was used as the substrate, which modified protocol from Nurrochmad (Nurrochmad *et al*., 2018). The extract (20 µL) was added to 100 µL of 0.1 M phosphate buffer (pH 6.8) and mixed with 40 µL of 1 mM L-DOPA in 0.1 M phosphate buffer (pH 6.8). The mixture was incubated at 37ºC for 10 minutes. Then, 40 µL of 200 unit/mL tyrosinase in 0.1 M phosphate buffer (pH 6.5) was added and incubated at 37ºC for 15 minutes. The absorbance was measured at 475 nm. Anti-tyrosinase activity was calculated as follow:

$$\text{Tyrosinase inhibition (\%)} = [(A_{control} - A_{sample})/A_{control}] \times 100 \quad (3)$$

Where $A_{control}$ is the absorbance of the control (without extract) and A_{sample} is the absorbance in the presence of the test sample. Then, tyrosinase inhibition of extract was expressed as 50% inhibition concentration (IC_{50}) and kojic acid was used as the positive standard.

2.7 Cytotoxicity

The cytotoxicity assessment was performed by MTT assay (Im *et al*., 2003). $B_{16}F_{10}$ melanoma cells (melanocyte cells) were cultured in DMEM and supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin solution. Cells were maintained in 5% CO_2 at 37ºC in the incubator (Model CB 60 CO_2 incubators, Binder, Tuttlingen, Germany). The test was performed by seeding the cells in a 96-well plate at a density of $1x10^4$ cells/mL and treated with various extract concentrations (13-65 mg/mL) in media for 24 hours. Afterwards, the cell culture medium was removed and 5 mg/mL of MTT solution

(25 µL) was added and mixed with medium without supplement (175 µL) to each well. After being incubated for 4 hours, DMSO (150 µL) was added to all wells and incubated for 30 minutes at room temperature. The absorbance of each sample was measured at 570 nm using a microplate reader. The percentage of the viable cell was calculated by the following formula.

$$\text{Viable cell (\%)} = (A_{\text{treated group}}/A_{\text{untreated group}}) \times 100 \quad (4)$$

Where, $A_{\text{untreated group}}$ is the absorbance of the control (without extract) and $A_{\text{treated group}}$ is the absorbance in the presence of the test sample. The cytotoxicity activity of the extract was expressed as the 50% cytotoxic concentration (IC_{50}).

2.8 Formulation

The rhizome star grass extract cream was prepared according to the O/W emulsion as displayed in Table 1. The O/W emulsion formula is based on the research of Pueknang and Saewan (2022). In part A and B, all ingredients were separately weighed and heated to 70ºC, then part A was added into part B and mixed well by using a homogenizer (T25 digital ultra turrax, IKA, Staufen, Germany) for 15 min. Afterwards, the mixture was cooled down to 40ºC, and part C was added into part AB and homogenized for 5 minutes which obtained a cream base. Rhizome star grass extract at concentration of 3% was incorporated to prepared cream base, obtaining RSG cream. Both creams appeared no phase separation after centrifuged at 9,000 rpm for 30 minutes. The products were measured for a pH value by using pH meter (HI2211 HANNA Basic pH/ORP Benchtop Meter, Hanna Instrument, Bangkok, Thailand). Lastly, all products were evaluated for characteristics such as appearance, color, texture, and spreadability by visual observation and applying on the skin.

Table 1 Base and active creams formulation.

Part	Ingredients	%(w/w)
A	Deionized water	q.s. to 100
	Butylene glycol	2.0
	Glycerin	5.0
B	Glyceryl stearate	1.2
	Lexemul 561®	1.5
	Cetearyl alcohol	1.5
	Novemer EC-1 polymer®	1.5
C	Phenoxyethanol	0.8
	Rhizome star grass extract	3.0

2.9 Clinical Study

The skin irritation study was performed using patch testing (Queille-Roussel et al., 2001; Schnuch *et al*., 2008). The protocol was approved by the Ethics Committee of Mae Fah Luang University under protocol number EC-21062-17. All volunteers signed written informed consent before participating in the clinical trial. The inclusion criteria include men and women with normal skin, Fitzpatrick skin types II-IV (Fitzpatrick, 1988), and aged between 20-60 years and while the exclusion criteria were subjects who have a chronic skin condition, sensitive or easily irritated skin, pregnant, regularly smoked or drank alcohol, as well as those who had taken part in the same experiment within the previous month. Moreover, each participant was acknowledged about the type of study, the procedures to be followed, the general nature of materials being tested and any known or anticipated adverse reaction that might result from participation.

Thirty-two volunteers, 3 males and 29 females, were subjected to occlusive patch test by patch testing method. The four samples were included a positive control (0.2% sodium lauryl sulfate), negative control (deionized water), base cream, and active cream with 3% rhizome star grass extract. The positive and negative controls (0.015 mL) were soaked in a circular filter paper, and then contained in the Finn Chamber®. While both creams (0.1 mg) were filled in each chamber. The patch test was performed by attaching on the upper arm for 24 hours. After that, the grade scores of skin irritation (erythema and edema) were investigated using score criteria for dermal reactions after removing the

patch for 30 minutes and 24 hours. The result was reported as cumulative irritation index (C.I.I) for each subject to the following formula:

$$C.I.I = \sum \text{ of the grade (erythema + oedema) / number of readings} \quad (5)$$

This index is then divided by the number of volunteers to obtain the mean cumulative irritation index (M.C.I.I) by the following formula:

$$M.C.I.I = C.I.I / \text{number of volunteers} \quad (6)$$

The M.C.I.I value of tested samples should be less than 0.25 which considered as non-irritant, while scores greater than 0.26 regarded as irritation.

The participants who passed the occlusive single patch test without experiencing any irritation (M.C.I.I score <0.25) was able to take part in clinical study. To prevent bias, a number rather than a name was given to each volunteer. Two treatment groups were assigned randomly to volunteers. The first group (placebo group) received base cream (B cream), while the second group received cream with 3% rhizome star grass extract (RSG cream). All volunteers were instructed to apply the test product on the facial every day, which was used 2 times a day (in the morning and before night) for 8 weeks. Besides, the specific instructions for this experiment were not to use any other skincare products and should be avoided the sunlight in the relevant areas.

Before the assessment, each volunteer's face was cleansed with a mild facial cleanser. Then, they acclimated to the temperature-controlled room (22ºC) and relative humidity (45-60%). The skin melanin and erythema contents and skin hydration were measured in triplicate at baseline (week 0), and week 2, 4, 6, and 8 after application, by using a single-blind method. The melanin and erythema contents of the skin were measured using Mexameter® (MX18, Courage Khazaka electronic, Cologne, Germany). The skin hydration was measured by using Corneometer® (CM 825, Courage Khazaka electronic, Cologne, Germany). All measured values were processed with Multi Skin Test Center® MC 1000 & Software and calculated as percent of change by the following equation:

$$\% \text{ Change} = [(X_t - X_0)/X_0] \times 100 \quad (7)$$

Where X_0 is the initial value measured before application (week 0) and X_t is the value measured during application (Fox *et al*., 2014).

2.10 Statistical Analysis

Statistical analysis was performed by using IBM SPSS Statistic Program (SPSS Inc, Chicago, IL, USA). Continuous variables were expressed as mean±standard deviation (SD) representing data as triplicate values. The experiment of TPC, DPPH, FRAP, and anti-tyrosinase activity was carried out using One way analysis variance (ANOVA), while clinical trial values were performed using the paired t-test. The significant difference was considered when the p-value was below 0.05.

3. Results and Discussion

3.1 Extraction

Fresh rhizomes of star grass were long in shape, with dark brown bark, and internal white wood (Figure 1A), and when they dried, appeared slightly darker (Figure 1B). After blending, the purple powder was obtained (Figure 1C) with a percentage yield of 47.6±2.0%. The star grass rhizome extracts with various solvents of water, 50% and 95% ethanol by ultrasonic extraction were shown in Figure 2. The extraction with the higher polarity solvent was obtained as a darker brown solution due to the rhizomes of star grass containing pigments that aqueous solvent could dissolve pigment greater than ethanol solvent. All extracts were evaporated to remove the solvent and kept at 4ºC before analysis.

3.2 Total Phenolic Content

The phenolic compounds have been widely reported and proved to be gainful to humans who have a potent for antioxidant, antimicrobial, and anti-inflammation. The antioxidant can prevent skin ageing and damage, including wound and burn. Furthermore, phenolic compounds also prevent the progression of certain skin disorders like acne (Yao *et al*., 2004; CevallosCasals & Cisneros-Zevallos, 2010; Dzialo *et al*., 2016). The phytochemicals contents in the star grass extracts were consisted of alkaloids,

saponins, flavonoids, anthocyanins, glycosides, and tannins, according to literature of Manosroi (Manosroi *et al*., 2012).

The aqueous extract (0.43±0.01 mg GAE/mL) showed significantly higher total phenolic content (TPC) than 50% and 95% ethanolic extract (0.20±0.02 and 0.05±0.01mg GAE/mL, respectively) as shown in Table 2. The results indicated that different polarity solvents had a significant effect on polyphenol contents in star grass extracts. Additionally, the result corresponded to the study of Boonpisuttinant, who reported that aqueous extract of star grass from tuberous provided the highest TPC contents compared to ethanol extracts (Boonpisuttinant *et al*., 2013).

3.3 Antioxidant Activities

The antioxidant activities of the samples were investigated with two different methods, DPPH and FRAP activities, which shown in Table 2. According to literature of Clarke *et al*. (2013), the screening of plant activity by analyzed several methods and combining the data could provide a better description of antioxidant activities than data obtained from a single assay. DPPH radical scavenging is one of the most common assays for evaluating samples' ability to scavenger free radicals. This assay determines the electron donor ability of samples, resulting in the reduction of a purple DPPH radical that can stabilise the molecule. Whereas FRAP assay was based on the reduction of ferricyanide complex (Fe^{3+}) to the ferrous form (Fe^{2+}) (Huang *et al*., 2005; Alam *et al*., 2013).

The 50% ethanolic extract showed the lowest of 50% inhibitory concentration of extract (IC_{50}) value of 0.58±0.00 mg/mL. However, it was no significant difference when compared with aqueous extract (IC_{50} of 0.59±0.02 mg/mL) and 95% ethanol extract (IC_{50} of 0.61±0.01 mg/mL). All extracts exhibited about 5 times higher IC_{50} value than standard ascorbic acid (IC_{50} of 0.11±0.25 mg/mL).

As well as the FRAP results, all solvents demonstrated a similar value of FRAP activities without significant differences. The rhizome star grass extract in 50% ethanol gave the highest FRAP value (0.18±0.01 mg AAE/mL). The water extract presented as the second good activity (0.16±0.03 mg AAE/mL) and followed by 95% ethanol extract (0.14±0.02 mg/mL). The results obtained were in the same direction as previous literature, which apprised that star grass tuberous in aqueous extract offered higher antioxidant activity than ethanolic extract (Boonpisuttinan *et al*., 2013). Interestingly, it was found that the combination of two solvents in the appropriate ratio, like 50% alcohol, provided higher antioxidant activities than using a single solvent.

A B C

Figure 1 Appearance of fresh (A), dried (B), and powder (C) star grass rhizomes

A B C

Figure 2 The extracts of star grass rhizome by water (A), 50% ethanol (B), and 95% ethanol (C)

3.4 Anti-Tyrosinase Activity

Reactive oxygen species (ROS) are play an important role in the regulation of the melanogenesis that contributes to stimulate signs of aging include dark spots, melasma, freckles, and even hyperpigmentation syndromes, whereas antioxidants can interact with the stabilize free radicals and prevent melanogenesis which associated to inhibition of tyrosinase (Briganti *et al*., 2003; Choi & Shin. 2016).

The 50% ethanolic extract of star grass rhizome indicated a significantly highest inhibition of tyrosinase activity than water and 95% ethanol extract (p<0.05), data was shown in Table 2. Although this extract was less efficient than kojic acid (IC_{50} of 0.13±0.46 mg/mL). The result suggested that 50% ethanol was the best solvent which responded to anti-tyrosinase activity and also in line with antioxidant activities. This resultant was corresponding to previous literatures (Cheng *et al*., 2009; Boonpisuttinant *et al*., 2014) which explained that main phytochemical contents in star grass like tannin, glycoside, aureaside A, and aureaside B exhibited antioxidant and inhibition of tyrosinase activities and had similar efficacy when comparison to vitamin C. However, according to Park (Park *et al*., 2003) reported that kojic acid exhibited a high tyrosinase inhibition efficiency which is equivalent to hydroquinone, while vitamin C presented a lower inhibitory. Consequently, in this research, kojic acid was selected as standard for determination. Moreover, the semi-polar solvents were substantiated as the most suitable for extracting tannin and quercetin which are the major compounds found in star grass (Zhong-Quan *et al*., 2011; Neelapong *et al*., 2018). Thus, star grass rhizome extract in 50% ethanol was selected for further study.

Table 2 TPC, antioxidant activities, and anti-tyrosinase activity of the star grass rhizome extracts.

Extracts	TPC (mg GAE/ml)	DPPH activity (IC_{50} mg/mL)	FRAP activity (mg AAE/mL)	Anti-tyrosinase (IC_{50} mg/mL)
Water	0.43±0.01^{b}	0.59±0.02^{b}	0.16±0.03^{b}	1.92±0.02^{c}
50% ethanol	0.20±0.02^{c}	0.58±0.00^{b}	0.18±0.01^{b}	0.68±0.01^{b}
95% ethanol	0.05±0.01^{d}	0.61±0.01^{b}	0.14±0.02^{b}	4.84±0.23^{d}
Gallic acid	3.79±0.20^{a}	-	-	-
Ascorbic acid	-	0.11±0.25^{a}	3.82±0.12^{a}	-
Kojic acid	-	-	-	0.13±0.46^{a}

Values are presented as means±SD from triplicate.
Data with different letters (a, b, c, and d) in the same column indicate significant differences (p<0.05).

3.5 Cytotoxicity

The 50% of star grass rhizome extract at concentrations 13, 26, 39, 52, and 65 mg/mL were investigated for cytotoxicity to $B_{16}F_{10}$ melanoma cells by MTT assay (Figure 3). The extract at low concentrations of 13 and 26 mg/mL showed the higher viable cell with 130.73±3.42% and 109.39±4.6%. Whereas the higher concentration at 39, 52, and 65 mg/mL of the extract showed lower cell viability of 75.38±3.06%, 21.16±1.60%, and 17.05±1.29%, respectively. The concentration causing 50% viable cell (IC_{50}) of the extract was 47.54 mg/mL. When considering the cell viability more than 80%, it was found that the maximum concentration not exceeded at 26 mg/mL could be used in cosmetic formulation. However, human skin is different from the cells. The skin acts as several barrier functions, such as durable diffusion of chemicals when directly in contact to the skin, against UV radiation and microbes that are termed as skin barriers, unlike the cells extremely subtle (Lodén, 2016). Hence, the star grass rhizome extract could be used in higher doses.

3.6 Formulation

The topical base cream and active cream, which incorporated 3% of star grass rhizome extract, were developed to give a good texture, easy to spreadability and absorb, and non-sticky. The appearance of both creams is displayed in Figure 4. The prepared base cream (B cream) appeared white texture, while rhizome star grass cream (RSG cream) obtained light brown texture. The finished products had a pH range of 5.70 to 5.90.

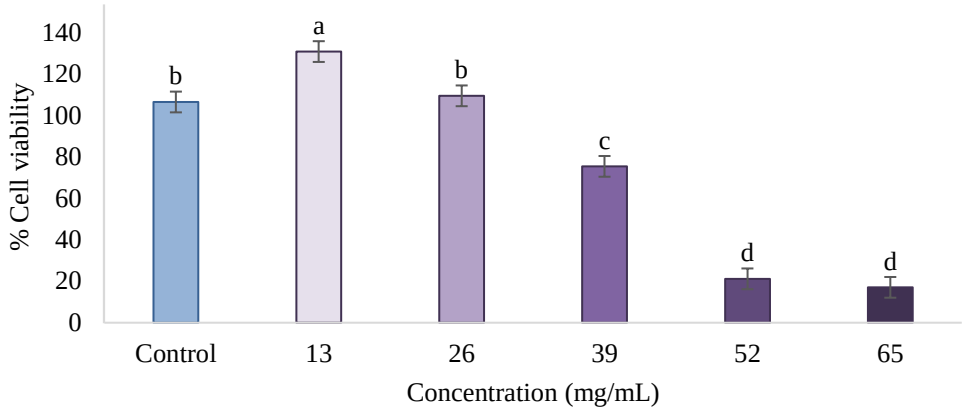

Figure 3 The percentage of cell viability on $B_{16}F_{10}$ melanoma cells of star grass rhizome extracts. Values are presented as means±SD from triplicate. Data with different letters (a, b, c, and d) indicated significant differences (p<0.05) when compared to control and various concentration of extracts.

A B

Figure 4 The appearance of B cream (A) and RSG cream (B)

3.7 Clinical Study

The skin irritation test was investigated by using an occlusive single patch test. The volunteers were assessed for the erythema and edema on the upper arm after 30 minutes and 24 hours patch removal. The grade of skin irritation was recorded as C.I.I value for each volunteer. Then, the results were calculated and concluded as the M.C.I.I value. Sodium lauryl sulfate showed M.C.I.I values of 0.81 and 0.62 after 30 minutes and 24 hours removing patch which classified as slightly irritation, while the M.C.I.I. values of deionized water and B cream were 0.00 which assessed as non-irritation. The RSG cream exhibited M.C.I.I values of 0.09 and 0.03 after 30 minutes and 24 hours patch removal which classified as no irritation. Besides, all test samples showed no signs of edema (M.C.I.I value of 0.00) in all periods of time after remove the patches.

The clinical study of brighten, redness, and hydration effects of RSG cream were evaluated on facial skin on 28 volunteers, 2 males and 26 females, compared with B cream (placebo) for 8 weeks. The evaluation of skin brightening by measured the melanin content using Mexameter®. The melanin content value can distinguish between skin darker and lighter. The results showed that B cream was not any change. In contrast, the RSG cream slightly decreased of melanin contents after 2 weeks and 4 weeks of application and significantly demonstrated reduction during 6 and 8 weeks (p<0.05). After 6 and 8 weeks, could lower the melanin level by 6.68±0.30% and 6.87±0.28% compared to the initial value (Figure 5A). These results corresponded to anti-tyrosinase activity of 50% ethanolic star grass rhizome extract and to the anti-melanogenesis of tubers and leave star grass in water and alcohol extracts, according to Boonpisuttinant *et al*., (2014).

The RSG cream also provided redness reduction ability, which was performed by measurement of erythema content using Mexameter®. Redness on the face is one of the most easily recognizable clinical signs which causes by many factors such as skin inflammation, allergies, and photodamage. A red face may be due to transient erythema (flushing) or persistent erythema. Flushing may be precipitated by

sunlight, a hot and humid atmosphere etc. In recent years, many topical formulations with rich antioxidants have been used for reducing redness in human skin (Logan, 2013; Dessinioti & Antoniou, 2017; Siu *et al*., 2017). At weeks 6 and 8, the erythema contents had dramatically decreased from the beginning with 6.90±0.40% and 6.97±0.38%, respectively (p<0.05). Whereas B cream was not any change values (Figure 5B).

Skin hydration is related to water content in the stratum corneum, the outermost layer of the epidermis. It plays several important roles in skin function, including cell proliferation, moisture, skin barrier, and anti-inflammation. Additionally, the products that can enhance skin hydration will prevent dryness and even premature aging (Verdier-Sévrain & Bonté *et al*., 2007; Spada *et al*., 2018).

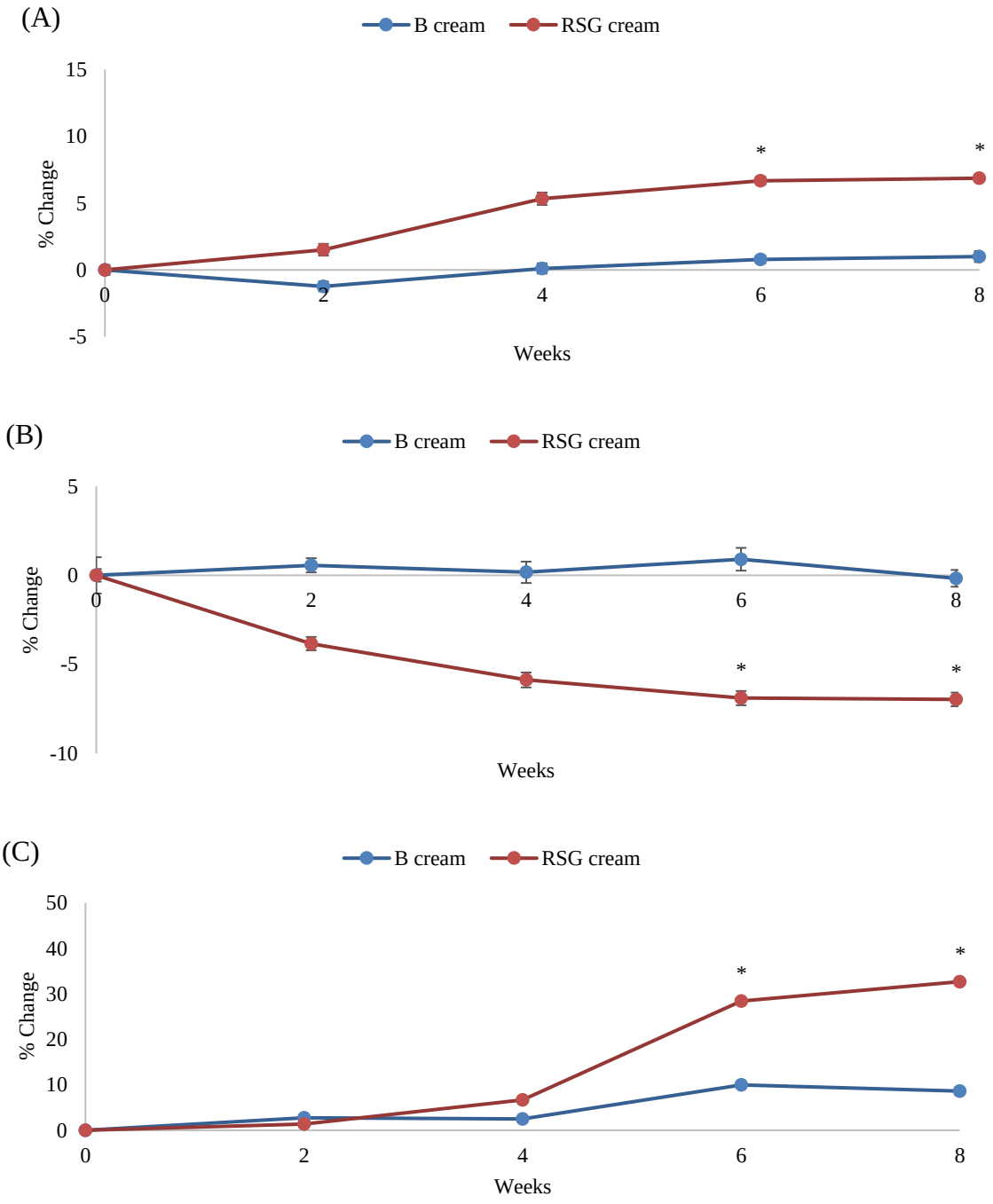

Figure 5 Percentage of change in skin brightening (A), redness (B), and hydration (C) of all volunteers after applied products. Data with * indicate significant differences (p<0.05) of each product after application compared to baseline.

According to Figure 5C, the percentage change of skin hydration of subjects who applied RSG creams was significantly increased by 28.40±0.35 for week 6 and continuously enhanced to 32.70±0.26% at week 8 (p<0.05). The placebo group, which used B cream, also found that the hydration was increased, however, till week end, base cream could promote hydration of 8.62±0.91% without significant difference. The reason of B cream demonstrated the improved skin hydration due to glycerin and butylene glycol that incorporated in the formulation, which had humectant abilities and might fulfil moisture during application.

Overall, the star grass rhizome extract was suggested to be an effective active ingredient for use as skin brightening, reduction of skin redness due to anti-inflammation, and skin hydration which application in cosmetics and cosmeceuticals.

4. Conclusion

The aqueous extract of dried star grass rhizome showed the highest TPC (p<0.05). The DPPH and FRAP activities of water, 50% and 95% ethanol extract exhibited no significant differences. The anti-tyrosinase activity of 95% ethanolic extract significantly demonstrated the highest activity (p<0.05). It was found that semi-polar solvent appropriate for extracting rhizome star grass. The extract of 50% ethanol at a concentration of 26 mg/mL proved to promote cell viability up to 100%. Afterwards, 3% of the extract was incorporated into the prepared base cream and obtained a light brown texture. The occlusive single patch test of rhizome star grass cream (RSG cream) showed M.C.I.I value of 0.09 and 0.03, which are classified as no skin irritation products. The clinical results showed that topical application of RSG cream suggests skin lightening, reduced redness, and enhanced hydration benefits. Statistical improvements were indicated in its ability to reduce the melanin and erythema contents and increase of skin moisture against the baseline. Thus, star grass rhizome extract is an effective ingredient for a skin brightening, anti-inflammation, and moisturizing agents.

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Effect of the types and concentrations of film-formers and concentrations of plasticizer on peel-off mask formulations

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Abstract

Peel-off masks are one type of cosmetic products commonly applied on the skin by leaving over time. They can form a thin plasticized film that can be peeled off. The main ingredients of peel-off mask formulation include polymers as film-formers, plasticizers, and moisturizers. In this study, the effect of film-formers, namely polyvinyl alcohol (PVA) and polyvinylpyrrolidone (PVP), at 5 or 10 %w/w, and plasticizer, namely butylene glycol, at 5 or 10 %w/w were determined in the gel bases. Appearance, pH, viscosity of gel, drying time, and swelling of the films were used as criteria for selecting suitable formulations. The result showed that the peel-off mask gels were clear, soft, smooth, and easy to apply. The pH of the gels decreased in the presence of film-former, compared to the control, and pH was significantly affected by the film-former concentration (p<0.001). All formulations had pseudoplastic (non-Newtonian) flow behavior which was suitable for topical application. The viscosity was affected by concentrations of film formers. It increased when the concentration of film former was increased. Both PVP and PVA transformed gels into films after drying, but PVA offered more stretchable and continuous films. In terms of drying time, PVA gel dried and formed films faster than PVP gel. The concentrations of film-former significantly reduced the drying time (p<0.001). The swelling of the polymeric films was evaluated by measuring the weight increase after adding water on the dried films. The swelling ratio was decreased when the film-former was included, and the higher the concentration, the lower the swelling ratio. The types of film-formers did not affect the swelling ratio significantly (p=0.995). On the other hand, butylene glycol had minor influences on gel and film characteristics. In conclusion, PVA and PVP in peel-off mask formulations affected the gel and film characteristics differently. The concentrations of film-formers had significant effects on pH, gel viscosity, drying time, and swelling of the films, while the concentration of butylene glycol slightly affected the gel and film properties.

Keywords: Polyvinyl alcohol; Polyvinylpyrrolidone; Butylene glycol

1. Introduction

Cosmetic facial masks come in various forms, such as sheet masks, rinse-off masks, peel-off masks, and hydrogel masks and they are formulated as gel, emulsion, paste or sheet (Nilforoushzadeh *et al.*, 2018). The peel-off mask gels, one of the most popular forms, can form a film on the skin within 15-30 minutes after application and the film can be removed without leaving any residue. Peel-off mask gels are beneficial for deep pore cleansing, moisturizing, enhancing the occlusive effect, improving blood circulation, removing dead skin cells, and softening the skin (Rahmasari *et al.*, 2019).

Mainly, the peel-off mask gels are composed of film-former, plasticizer, and moisturizer. However, most research studied only one type of film-former and a single concentration of plasticizers. There is no information about a comparison of types of film-former or concentrations of plasticizers to determine

whether film-formers or plasticizers are appropriate for product development, as in the previous research (Rahmasari *et al.*, 2019).

Polyvinyl alcohol (PVA) is a water-soluble synthetic polymer, and functions as a binder, film former, and viscosity-increasing agent-aqueous in cosmetic products. The PVA gels are dried easily, form a strong transparent film and adhere well to the skin (Hariyadi *et al.*, 2021; Nair *et al.*, 1998). Polyvinylpyrrolidone (PVP) is a typical hydrophilic and synthetic polymer, pH-stable, nontoxic, and biocompatible with excellent film-forming ability. PVP has been used in medicine, pharmaceuticals, cosmetics, and foods (Nešić & Ružić, 2017). Butylene glycol (BG) is non-toxic, colorless, and is used as a humectant and plasticizer for polymers (Lieber, 1985). Thus, these ingredients would be used in our study.

The purposes of this study were to compare the type and the concentration of film formers (PVA or PVP), and the concentration of plasticizer (BG). Organoleptic tests including pH, viscosity, film drying time and film swelling were evaluated.

2. Materials and Methods

2.1 Material

Polyvinylpyrrolidone K90 (PVP-K90, molecule weight 90,000 Daltons) was purchased from Chanjao Longevity Co., Ltd. (Bangkok, Thailand), Polyvinyl alcohol (PVA, Mowiol® 18-88, molecule weight 130,000 Daltons) was purchased from Sigma Aldrich (Missouri, USA). Butylene glycol was purchased from Chanjao Longevity Co., Ltd. (Bangkok, Thailand). Other chemicals in gel formulations were cosmetic grade.

2.2 Preparation

The gel base contained some basic ingredients. Two gelling agents were dispersed in butylene glycol. The stabilizer, chelating agent, whitening agent, and preservative were dissolved in purified water before the mixture of gelling agents was added, homogeneously. Moisturizer and PVP-K90 or PVA were added to the gel at the final step. The concentrations of PVP-K90, PVA, and BG in the formulations were 5 or 10 %w/w.

2.3 Method

2.3.1 Physical appearance and pH

The physical appearance was seen as its color and consistency visually. The pH value was determined by using a digital pH meter. (S220, Mettler Toledo, USA). The results were expressed as the mean ± SEM of three measurements.

2.3.2 Viscosity

Rheology measurement was made using a Rheometer (HAAKE MARS 40/60, Thermo Scientific, USA). Data analysis was made using Haake Rheo-Win. The spindle specification was a parallel plate (C60 1◦/Ti-02180278). The temperature of the gels were set constant at 25 ± 0.20 °C (KASSAB *et al.*, 2017). The shear rate ranged from 0.1 to 100 s^{-1}. The graphs were then presented as shear stress (τ) to the function of shear rate ($\dot{\gamma}$). The results were expressed as the mean ± SEM of three measurements.

2.3.3 Film formation and drying time

Film drying time was evaluated by a hot air oven and on the skin. Approximately 0.2 g of each formulation was spread over a glass plate of 2x2 square centimeters forming with a thickness of approximately 2.0 millimeters. The glass plate was heated in the hot air oven (37.0 °C) to simulate skin temperature (Vieira *et al.*, 2009). For the evaluation on the human skin, 0.2 g of each formulation was applied over the researcher's skin (n=1) of 2x2 square centimeters, forming a uniform mask layer with a thickness of approximately 2.0 millimeters. The researcher stayed at room temperature (25.0 °C, 65% RH) to simulate virtual reality. The end point of drying time was when non-sticky film surface was observed by touching. The results were expressed as the mode of three measurements.

2.3.4 Swelling of the film

The swelling of the polymeric films was evaluated by measuring the weight increase after adding

water on the dry film. Approximately 0.2 g of each formulation was distributed on a 2x2 cm^2 glass sheet. The glass plate was placed to heat in the hot air oven (40.0 ℃). Samples of each film were weighed. The film was then placed on a glass slide, the water was dropped on the film, the excess water was wiped with blotting paper, and the swollen film was weighed (Peh and Wong, 1999). The results were expressed as the mean ± SEM of three measurements. The degree of fluid uptake was calculated as a swelling index using the following equation:
swelling index = (final weight - initial weight)/ initial weight) ×100

2.3.5 Statistical analysis

All results were analyzed with the IBM SPSS statistics program (version 28). Data and statistical analysis were done by using the one-way analysis of variance (one-way ANOVA) Post Hoc Tests, Tukey HSD. A value of $p < 0.05$ was considered statistically significant.

3. Results and Discussion

3.1 Physical appearance and pH

The physical appearance included the color, and consistency of the preparation. The appearance of the formulation is demonstrated in Figure 1. All peel-off mask gels had no color and semi-solid consistency. The texture of the gels weas stretchy, soft, smooth, and easy to apply.

The pH of the formulation is shown in Table 2. The pH value should be in the broad range of skin pH (from 4.0 to 7.0) to avoid any irritation to the skin and the pH of our peel-off mask gel preparations was approximately 5 (Ali and Yosipovitch, 2013). Therefore, the pH of peel-off mask gel preparations was within the optimal pH range of the skin. Different concentrations of film-former and butylene glycol contributed to significantly different pH (p<0.01). When the film-former concentrations were increased, the pH was lower than the control because the pH of the film-former was around 5.0, whereas the gel base had a pH of 5.0-6.0.

3.2 Viscosity

All the gel formulations demonstrated the non-Newtonian shear-thinning pseudoplastic type of flow. A typical example of the flow curve of the gel formulations is shown in Figure 2. The viscosity of all gels decreased concerning the applied shear rate, which caused better skin spreadability.

The viscosity of each formulation was measured using a Thermo Scientific Rheometer at shear rates of 1 and 10 s^{-1}, where 1 s^{-1} was a representative of self-leveling and 10 s^{-1} was a representative of spreading force on the skin. The viscosity of the formulation was shown in Figure 3. Different types of film-formers produced not significantly different results (p=0.658 to 1), but formulations compared to the control had significantly different results (p <0.01). This means that adding a film-former to the formulation had an effect on viscosity because PVA and PVP were viscosity-enhancing (Nair B., 1998). PVA10/BG5 and PVA10/BG10 have the highest viscosity. As a result, increasing the concentration of film-former produced significantly different results (p<0.01) similar to other studies (Sato and Kohnosu, 1994; Yuliani, 2010).

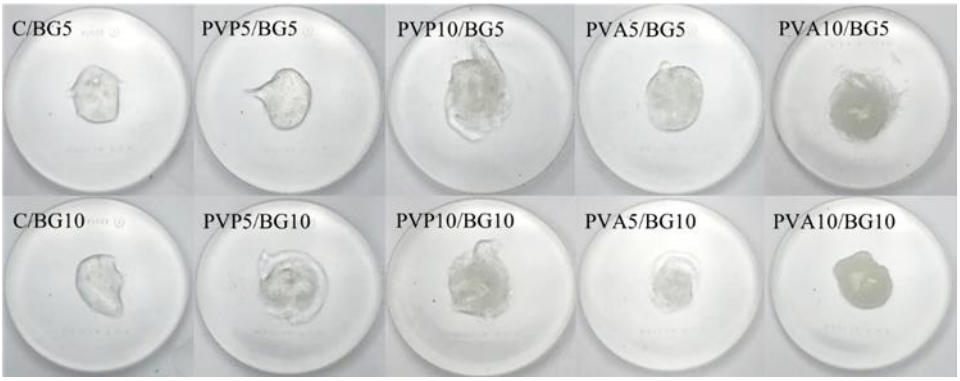

Figure 1 Gel appearance on transparent glass (C – Control group: gel base without film former)

Table 2 pH of peel-off mask gels

Formulation	pH (mean ± SD)
C/BG5	5.54±0.02
PVP5/BG5	5.43±0.01
PVP10/BG5	5.08±0.02
PVA5/BG5	5.44±0.03
PVA10/BG5	5.24±0.03
C/BG10	5.51±0.01
PVP5/BG10	5.22±0.01
PVP10/BG10	5.15±0.01
PVA5/BG10	5.27±0.01
PVA10/BG10	5.47±0.01

* Results are presented as (mean ± SD)

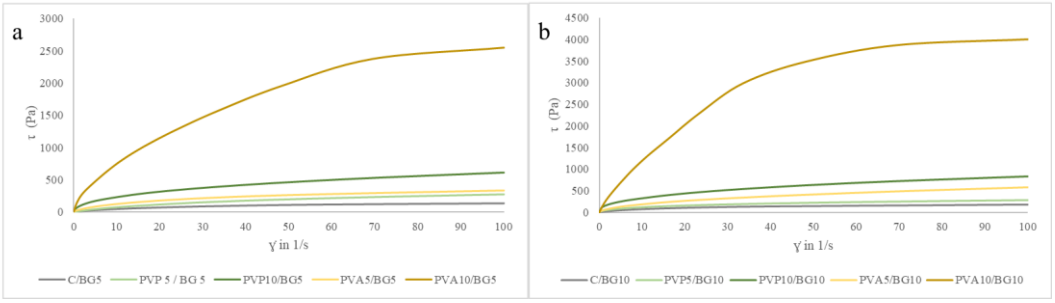

Figure 2 Shear rate vs shear stress plot of all formulations with (a) BG 5 or (b) BG 10

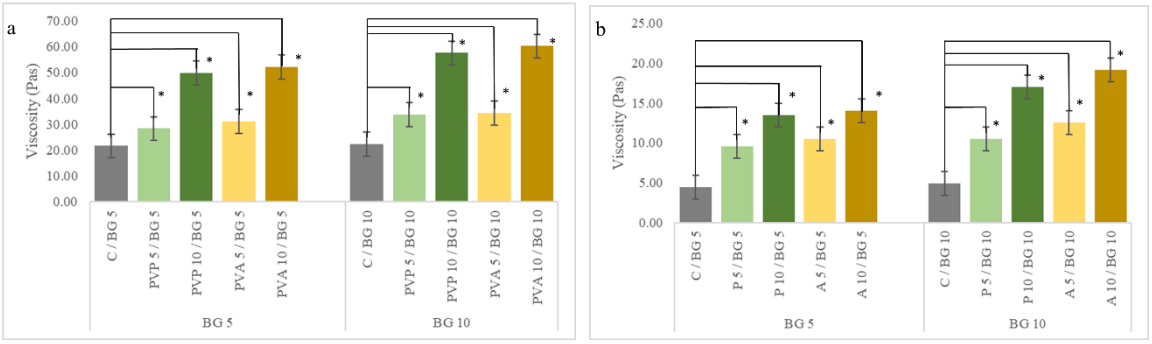

Figure 3 The viscosity of the formulation at (a) shear rate 1s-1 and (b) shear rate 10 s-1 *indicates a significant difference (p<0.05).

3.3 Film formation and drying time

The drying time of peel-off mask gels was one of the key criteria to identify the application time. The evaluation was performed on the researcher's skin in order to imitate the real application and to establish the setting for the hot air oven. The film formation characteristics from the researcher's skin of all formulations were shown in Figure 4. Both types of film-formers (PVP, PVA) could form film well but had different film characteristics. PVP provided a moist, flaky film, whereas PVA gave a dry, continuous film. In contrast, films from a gel base (control) created a wet, non-continuous film. As a result, the types of film-former influenced film characteristics.

Film drying time was evaluated by two methods: in the hot air oven and on the researcher's skin.

The evaluation from both methods was in the same direction. The drying time was shown in Figure 5. Ideally, the peel-off mask gels should dry between 15 – 30 minutes (Vieira *et al.*, 2009). However, the formulation in this study took longer than 30 minutes to dry, possibly because it did not use alcohol such as ethanol, as in other studies (Teng *et al.*,2010; André O'Reilly *et al.*,2013; Intan *et al.*, 2018). When the concentrations of PVP were increased, the drying time was increased, because PVP was known as a hygroscopic compound, which caused the increase of the water content in the peel-off mask gels, and as a result, the dry time of the preparation was longer (Teng *et al.*, 2010). On the other hand, When the concentration of PVA was increased, the drying time was decreased. The ability of PVA to attach to existing water molecules causes the attraction among water molecules and an increase in cohesiveness, then the dry time of the preparation was faster (Syamsuri and Isriany, 2021). Fast drying ability of PVA formulations was found in another study (Rahmasari *et al.*, 2019). The drying time of all formulations was a significant difference from the gel base without film former (p<0.01), but the different concentrations of BG did not cause any significant results (p=0.608 to 1).

3.4 Swelling of the film

The swell test was one of the numerous polymer capabilities that may be employed for various purposes, such as liquid absorption. The swelling index was calculated and shown in Figure 6. It can be seen that all formulations were capable of swelling because all formulations, including the control, consisted of gelling agents with excellent swelling capability. The control exhibited the highest swelling index due to the swelling ability of gelling agents in the gel base. However, when the film-former was added to the formula, the swelling index was significantly reduced (p<0.01). The film's swelling depended on polymer network's porosity and crosslinking density. When the polymer network increases, as the concentration of film-former increases, gel porosity and the ability to swell decrease (Gupta *et al.*, 2011; Gupta *et al.*, 2019). However, both the types of film-former and BG concentration produced insignificantly different results (p=0.06 to 0.995 and 0.924 to 1, respectively).

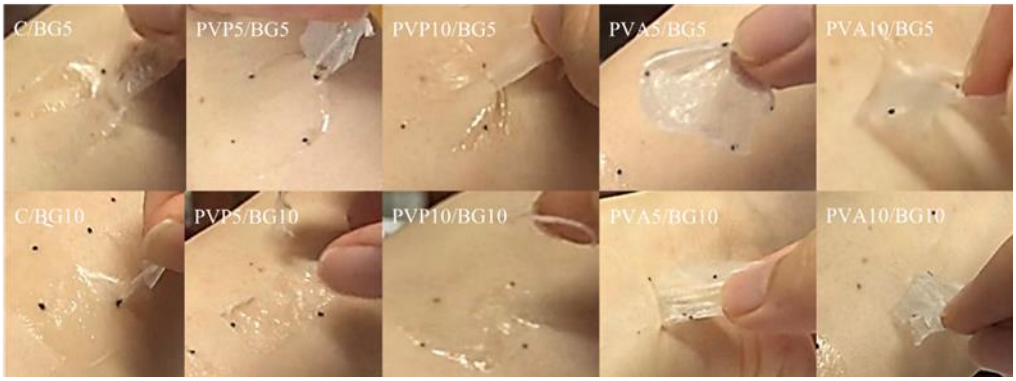

Figure 4 Film formation characteristics from the researcher's skin of all formulations.

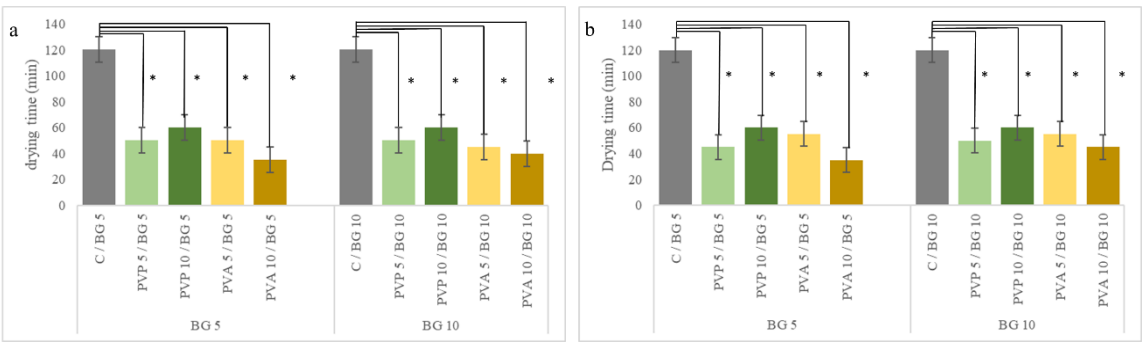

Figure 5 Film drying time of the formulation from (a) a hot air oven or (b) researcher's skin *indicates a significant difference (p<0.05).

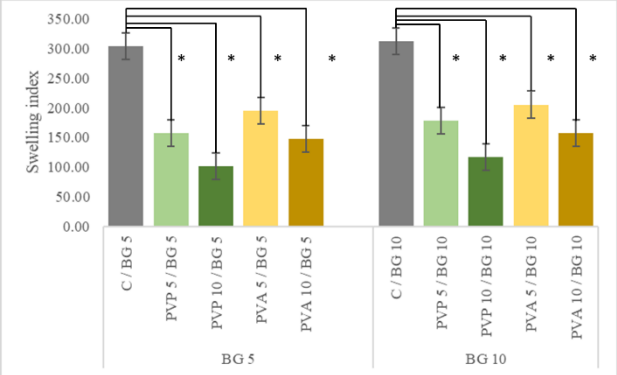

Figure 6 Swelling index of the formulations. *indicates a significant difference (p<0.05).

4. Conclusion

All formulations could form film for peeling masks. Adding PVA or PVP as a film former in the formulations provided different texture and film characteristics, and PVA demonstrated the continuous films which were suitable for our application. Different concentrations of film formers had a substantial influence on pH, gel viscosity, drying time, and swelling of the films. In contrast, butylene glycol concentration had little effect on gel and film characteristics.

5. Acknowledgements

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Formulation and stability testing of hydrating water containing protein hydrolysate from *Pleurotus ostreatus* (FR.) Guel.

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Abstract

Protein hydrolysate has been used in cosmetic products for more than 50 years. It has moisturizing properties and improved skin barrier. This research aimed to develop a hydrating water containing protein hydrolysate from *Pleurotus ostreatus* (FR.) Guel. The physicochemical properties and stability testing of the hydrating water were investigated. The hydrating water containing 0.05% and 0.20% protein hydrolysate was successfully developed as a clear yellow liquid, with a viscosity in the range of 1.30-1.40 cP. The moisture and rate of water loss of the skin were measured by Corneometer and Tewameter to compare these properties to the formulation without protein hydrolysate. Protein hydrolysate (0.20%) tended to increase skin moisture and reduce the rate of water loss from the skin after being applied for 60 min. A satisfaction survey of the formulation was tested with 30 healthy volunteers and the hydrating water containing 0.05% protein hydrolysate received the highest score. The physicochemical properties of this formulation were similar to the commercial product. The hydrating water containing protein hydrolysate was stable under 45±2°C, 75±5% RH for 1 month.

Keywords: Protein hydrolysate; *Pleurotus ostreatus* (FR.) Guel.; Hydrating water; Stability; Moisturizer; Satisfaction survey.

1. Introduction

Thailand is a country that has a favorable environment for the cultivation of mushrooms which are common fungi and are commonly consumed in Thailand. There are 13 types of economic mushrooms grown in Thailand, namely *Pleurotus pulmonarius*, *Pleurotus cystidiosus*, *Pleurotus ostreatus* (FR.) Guel, *Auricularia auricula-judae*, *Lentinus polychrous Berk*, *Lentinus squarrosulus*, *Lentinus edodes* (Berk.) Sing, *Coprinus fimetarrius*, *Cyclocybe aegerita*, *Calocybe indica* (P & C), *Schizophyllum commune*, *Ganoderma lucidum*, and *Cordyceps sinensis*. Mushroom production in Thailand has increased in Thailand over recent years. The Mushroom Researchers and Cultivation Association of Thailand found that in 2017, the amount of mushroom production was 41,322 tons, worth 2,291 million baht and in 2020, about 150,000 tons of fresh mushrooms were produced, worth 9,000 million baht (Buraso, 2017). However, there is an oversupply of mushrooms such as the shiitake mushrooms and the white log mushroom which are local mushrooms that are cultivated and have a high yield. This oversupply results in a substantial quantity of the harvest rotting, reducing the incomes of the growers. Therefore, other uses of both types of mushrooms are needed to add value to growers, to get the most benefit from mushroom growing (Heldreth, 2012; Scibisz et al., 2008; Wu et al., 2016). The cosmetic market is this other outlet.

Apart from their tastiness as a culinary ingredient, mushrooms contain valuable cosmetic bioactive ingredients, particularly protein which is one of the main components of the mushroom. Proteins and their hydrolysates from natural sources are commonly used as ingredients in hair and skin

care products (Aguirre-Cruz et al., 2020; Daithankar et al., 2005; McCarthy et al., 2013; Schrieber & Gareis, 2007). Proteins hydrolysates slowed down the deterioration of the skin because it increasing the moisture content of the epidermis and stratum corneum (Chang & Miles, 2004). It could reduce inflammation by reducing the TNF-α level (Manzi & Pizzoferrato, 2000). Moreover, the protein hydrolysates increase the strength of the skin by providing viscoelasticity and protecting skin aging (Sujarit et al., 2021). Hence, one method that can increase the value of mushrooms is by processing them into protein hydrolysate by extracting and undergoing hydrolysis at the peptide bond region containing short-chain peptides and free amino acids. Processed protein hydrolysate has high bioavailability in cosmetics such as the long chain protein converting to a smaller peptide and amino acid which have a smaller molecular weight, higher solubility, and higher absorption capability (Scibisz et al., 2008). Protein hydrolysates are soluble in water, non-toxic and dermatologically safe, providing good biological and moisturizing properties for the skin.

The objectives of the present research were to develop a hydrating water containing protein hydrolysate from *Pleurotus ostreatus* to serve as a moisturizer and to test its stability. A cohort of 30 volunteers participated in the program and responded to a satisfaction survey carried out using a questionnaire.

2. Materials and Methods

2.1 Materials

Protein hydrolysate from *Pleurotus ostreatus* (PHP) extracted with proteolytic enzymes to produce peptides below 3 kDa, was obtained from the Thai Mushrooms Hydrolysate laboratory of the Faculty of Medical Science, Naresuan University, Thailand. This laboratory researches skincare product development in association with 5 Phitsanulok mushroom farms. Butylene glycol, polyethylene glycol 400 and glycerin were obtained from Chanjao Longevity Co., Ltd, Bangkok, Thailand. phenoxyethanol was obtained from Sharon Laboratories Ltd., Ashdod, Israel and potassium sorbate was obtained from BASF (Thai) Ltd., Bangkok, Thailand.

2.2 Preparation of hydrating water formulations

Hydrating water formulations were prepared by solubilizing and mixing hydrophilic ingredients in a DI water. The mixer was firstly prepared by solubilizing potassium sorbate in water, and butylene glycol, polyethylene glycol 400, glycerin. PHP and phenoxyethanol were added and the solution was then homogenized. All 3 formulae had different %w/w of ingredients (Table 1). Hydrating water without PHP was prepared as a placebo. Commercial product containing protein hydrolysate from mushrooms (Formulation D) were purchased from a hypermarket.

2.3 Characteristics of hydrating water

The pH value, clarity and sedimentation of the hydrating water were observed by visual observation. The viscosity of the hydrating water was measured (CP-40, 50-200 rpm) by a viscometer (Brookfield Engineering Laboratories, Middleboro, MA).

Table 1. Ingredients and functions of substances in the hydrating water

	Formulation (%w/w)			
Ingredients	**F1**	**F2**	**F3**	**Function**
DI water	94.35	90.45	90.45	Solvent
Butylene glycol	2.00	4.00	4.00	Humectant
Polyethylene glycol 400	2.00	4.00	4.00	Humectant
Glycerin	1.00	1.00	1.00	Moisturizer
Phenoxyethanol	0.50	0.50	0.50	Preservative
Potassium sorbate	0.10	0.10	0.00	Preservative
PHP	0.05	0.05	0.05	Moisturizer

2.4 Stability Study of hydrating water

The stability of the hydrating water was studied by the accelerated method. The sample products were stored at 45 ± 2°C in 75 ± 5% relative humidity for 1 month. The physicochemical properties of the hydrating water products, such as pH value, clarity, and viscosity, were observed before and after testing, and the protein hydrolysate content of hydrating water was determined.

The Lowry assay (Bollag & Edelstein, 1991) was used for measuring the protein levels of the hydrating water in 96-well plates. After the preparation of Solution A (100 mL of distilled water with 5 g of $CuSO_4 \cdot 5H_2O$) and Solution B (1 L of distilled water with 21.2 g of Na_2CO_3, 4 g of NaOH), Solution C (1 mL of Solution A and 50 mL of Solution B) was prepared, followed by Solution D (10 mL of Folin-Ciocalteu phenol reagent and 10 mL of DI water). A 20 µL sample of mushroom protein hydrolysate was added to 200 µL of solution C and the solution was left at room temperature for 15 min. Then 20 µL of Solution D was added to the solution which was left for a further 30 min at room temperature. The absorbance of the sample was measured at 750 nm, using a microplate reader, and the protein concentration was estimated using a BSA standard curve from the same plate.

2.5 Study on skin moisturizing efficacy and transepidermal water loss (TEWL)

A 50 µL amount of each hydrating water formulation was dropped onto the forearm in a test area of 2×2 cm^2. At a predetermined time, skin moisture was determined using a Corneometer (Model CM 825, Courage+Khazaka electronic GmbH, Germany), and water loss from the skin was measured using a Tewameter (Model TM 300, Courage+Khazaka electronic GmbH, Germany). Each formulation was measured 8 times.

2.6 Satisfaction questionnaire study

In this study, a satisfaction questionnaire, which was approved by the Ethics Committee of Naresuan University Institutional Review Board, Naresuan University, Thailand (IRB No. P1-0081/2565), was given to each participant. The inclusion criteria for the study were that participants were healthy Thai females aged between 20 and 35 years. Thirty volunteers participated and their demographic information was recorded. Information on their cosmetic use was also ascertained. The information gathered from the satisfaction questionnaire was about formulation factors, including appearance, color, clarity, and attractiveness, as well as viscosity, of the product. For each question in the questionnaire, the answer choices were: excellent, moderate, poor, and bad. These choices were given a score of 4, 3, 2, and 1 respectively. The scores were averaged, and analyzed, to identify the shortcomings of the formulations to be resolved.

3. Results and Discussion

3.1 Development and characterization of hydrating water

To study the solubility of the PHP in the hydrating water formulations with different ratios of components were prepared. As can be seen in Figure 1, formulation F1 exhibited turbidity and agglomeration of proteins. Formulation F2 that contained a higher amount of co-solvent exhibited a higher level of protein agglomeration and turbidity than Formulation F1. Formulation F3, however, which was hydrating water without salt of organic acid preservatives, exhibited a clear yellow liquid with no agglomeration of proteins. These results suggested that potassium sorbate causes the salting out of PHP (Alvarez-Rivera et al., 2018). Hence, F3 was used as the starting formula in the development of hydrating water containing PHP, as illustrated in Table 2 where Formulation B contains 0.05% PHP and Formulation C contains 0.20% PHP. Formulation A, the control, contained no PHP.

Increasing the amount of PHP in the hydrating water caused a dark yellow color resulting from the yellow color of the PHP. However, no change in pH and no sedimentation were observed. Increasing the PHP concentration did not affect the viscosity of hydrating water because PHP contains short-chain peptides. All formulations exhibited Newtonian fluid flow meaning that the viscosity is constant and independent of the shear rate (Rehman et al., 2022).

3.2 Stability of hydrating water

A stability test was performed at accelerated conditions at 45 ± 2°C and 75 ± 5% RH for 1 month (Table 2). It was found that all three developed formulations did not change in terms of pH, color, clarity, and viscosity. According to a Lowry assay, the PHP content in B was 0.52 µg/µL and 1.38

Figure 1 Solubility of PHP in the hydrating water with different ratio of components

Table 2 Characteristics of hydrating water and stability testing after 45 °C for 1 month

	Formulation	A (without PHP)	B (0.05% PHP)	C (0.20% PHP)
Fresh preparation	Appearance			
	pH	7	7	7
	Color/clarity	Clear	Clear yellow	Dark yellow
	Sedimentation	no sediment	no sediment	no sediment
	Viscosity (cP)	1.35±0.04	1.46±0.01	1.38±0.03
	Protein content (ug/ µl)	-	0.52±0.01	1.38±0.03
45 °C for 1 month	Appearance			
	pH	7	7	7
	Color/clarity	Clear	Clear yellow	Dark yellow
	Sedimentation	no sediment	no sediment	no sediment
	Viscosity (cP)	1.36±0.05	1.49±0.02	1.29±0.01
	Protein content (ug/ µl)	-	0.58±0.01	1.40±0.01

µg/µL in C. After accelerated conditions, The PHP content did not change which suggests that PHP was stable in the developed hydrating water.

3.3 Study on skin moisturizing efficacy and transepidermal water loss (TEWL)

The efficacy of skin moisturizing of all formulations was measured using a corneometer and transepidermal water loss was measured using a Tewameter (Figures 2 and 3). The concentration of PHP was effective in increasing skin moisture and decreasing transepidermal water loss. This demonstrated that B and C are active moisturizers, similar to commercial products containing protein hydrolysate from mushrooms (D).

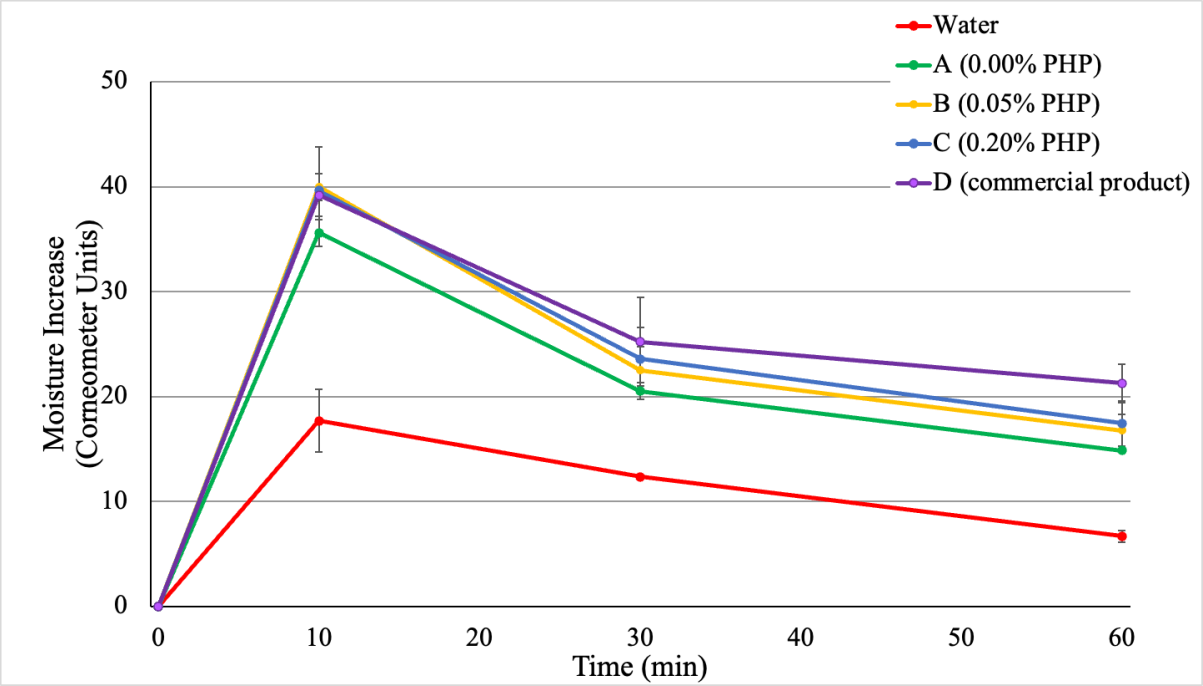

Figure 2 Effect of the formulation containing PHP on skin moisture following the application of the product at various intervals.

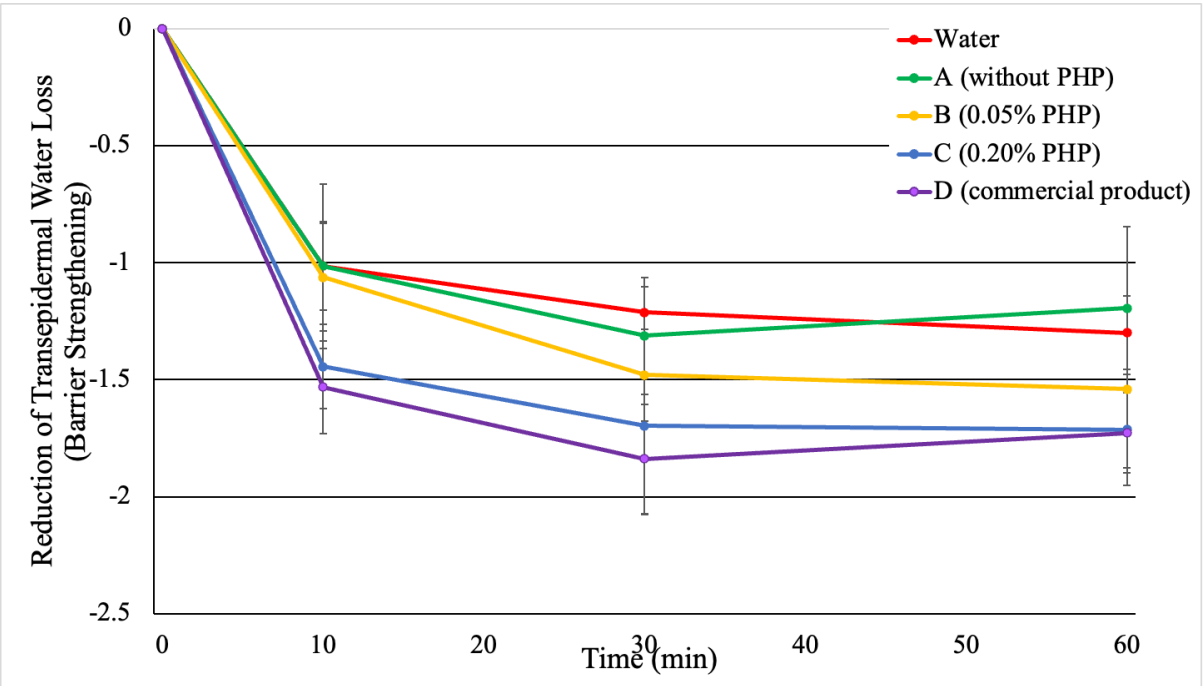

Figure 3 Effect of the formulation containing PHP on transepidermal water loss following application of the product at various intervals.

3.4 Volunteer satisfaction questionnaire of hydrating water

More than 70% of the volunteers (21 female volunteers) were 20-25 years old. They used the hydrating water daily. The product satisfaction data was a single-blind experiment in A, B, C, and D (Figure 4). Responses from the satisfaction questionnaire about the hydrating water products regarding the characteristics of the products indicated that the volunteers were more satisfied with the clear,

colorless hydrating water than with the clear formula that was slightly yellow. The volunteers had the highest satisfaction with the viscosity of D ahead of A then B and C. Because the volunteers were people who had already used hydrating water products in their daily lives, they were more familiar with commercial products than with the 3 developed recipes. The volunteers indicated that they were interested in B because it is clear, light yellow, not very dark, had low viscosity, was highly absorbed through the skin and not greasy. These characteristics made B more preferable than A, D and C.

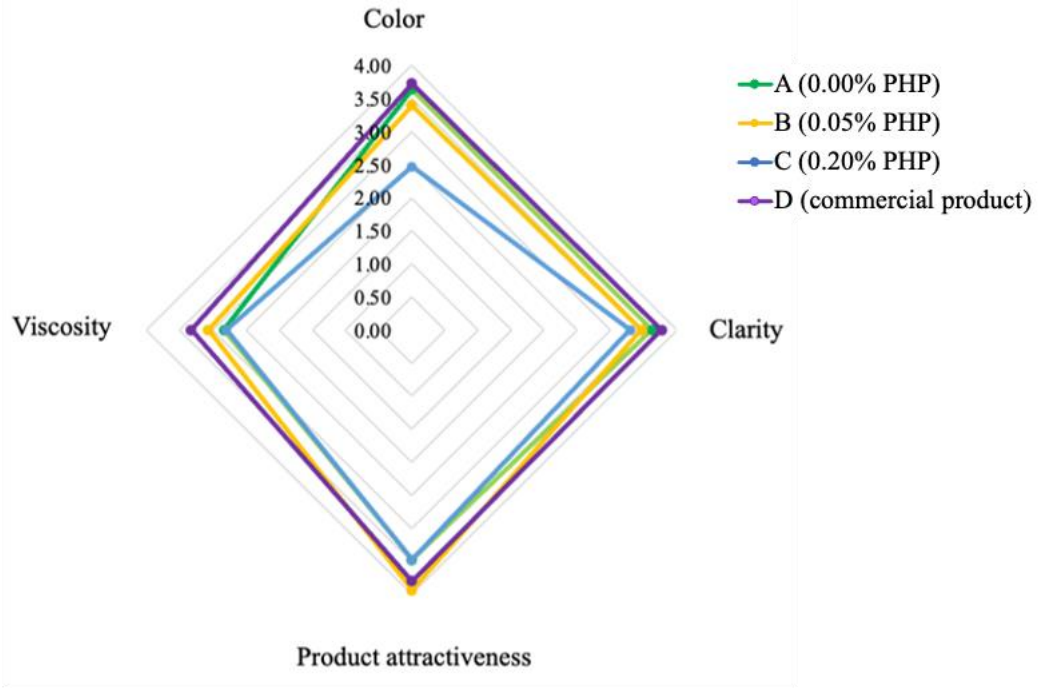

Figure 4 Product satisfaction assessment by single-blind experiment

4. Conclusion

PHP can be used as an effective ingredient and has promising potential in moisturizing effect and water loss prevention. The results confirmed that incorporating mushroom protein hydrolysate in the hydrating water enhanced skin moisture and decreased skin water loss. Satisfaction of volunteers on hydrating water results gave similar results that confirmed the effectiveness of cosmeceutical formulations containing PHP. Thus, we concluded that PHP is a valuable ingredient in cosmeceuticals, and we can use containing PHP to develop cosmeceutical products in various ways that are stable, pleasant in appearance, and provide great benefits to the dry skin.

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Screening evaluation of microbial contamination in expired and non-expired cosmetics used by female university students

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Abstract

Cosmetics have a great effect on female university students to make the appearance of attractiveness and confidence. Microbial contamination in some cosmetics with low-quality production can be found. The purpose of this study was to evaluate the microbial contamination in cosmetic products that are popular to use in female university students. This study included one hundred participants who are female university students at Mae Fah Luang University, Thailand. The participants were asked for providing general information, marking factors, and types of cosmetics that have usually been bought. Based on the cosmetics of the most favorite buying, four types of cosmetics with expired and non-expired dates including mascara, brush-on, lipstick, and foundation were chosen for the evaluation of microbial contamination. The serial dilution technique and swab test were used to prepare cosmetic samples and measure microbial colonies in the samples, respectively. The results showed that most expired and non-expired cosmetics were contaminated. The microbial contamination in the expired foundation and mascara was more than 1,000 cfu/g while that in the expired lipstick was less than 1,000 cfu/g. The expired brush-on did not have any microbial contamination. The microbial contamination in the non-expired foundation, mascara, and brush-on was found, but less than 1,000 cfu/g while that in the non-expired lipstick did not. The cell characteristics of microorganisms were observed by a light microscope. Most characteristics of microbial cells were expected to be bacilli and cocci. The results could be concluded that microbial contamination in the cosmetic products was likely to be found before and after expiration, but the expired products had a high risk to be contaminated extremely by microbes.

Keywords: Cosmetics; Expired products; Female teenager; Microbial contamination; Non-expired products

1. Introduction

Cosmetics means products prepared for use on the skin or any part of the body by using, rubbing, massaging, spraying, or sprinkling with the purpose of cleaning or making it beautiful or changing the appearance of that person (Mitsui, 1997). Cosmetics also have the effect of increasing the confidence or self-esteem of the user (Gillen & Dunaev, 2017). In addition, at present, cosmetics are popular with both men and women due to changes in lifestyle and values (Kumar *et al.*, 2014).

However, females still have a higher proportion of consumption of cosmetics than males, for example working women, the elderly, or teenagers, especially female students in university (Brown *et al.*, 1986). Due to these reasons, there are many types of cosmetics in the market that can be categorized follow by the FDA into 13 groups, including baby products, bath preparations products, eye makeup preparations products, fragrance preparations products, hair preparations products (non-coloring), hair coloring preparations product, make-up preparations product (Base make-up), manicuring preparations

product, oral hygiene product, personal cleanliness product, shaving preparations product, skincare preparations product (creams, lotions, powders, and sprays) and suntan preparations product (US FDA, 2022). Moreover, the quality and safety of cosmetics are very important and considered by consumers as the first priority of product production. Although there are a lot of cosmetics available for sale, sometimes some products, such as unsafe cosmetics or insufficient standard cosmetics, have been found. Microbial contamination is crucial one of the causative factors which usually result in the quality degradation of cosmetic products. Microbes that contaminate cosmetics may be opportunistic pathogens including non-pathogenic strains, e.g., *Staphylococcus aureas* this bacterium can produce filaggrin protein which can bind to the keratin fibers on the epidermis, helps to adjust fat levels, and maintain moisture (Reisch, 2017). The growth of microbes in cosmetics can cause many changes in the chemical characteristics and physical characteristics of the products resulting in feature changes in cosmetics such as stickiness, odor, color, and even phase separation and sedimentation. In addition, the regulations of Thailand in The Cosmetics Act B.E. 2558 state that cosmetics that have been opened and not opened must not detect pathogens are bacteria, fungi, and yeast, which are harmful to cosmetic users. For example, cosmetic products were contaminated with some pathogens such as *Pseudomonas aeruginosa*, which led to serious blindness (Wilson & Ahearn, 1977).

Nowadays, cosmetics have a great influence on teenagers because most teenagers choose to use cosmetics to decorate various parts of the body or to improve their appearance which cosmetics (Schiffman & Kanuk, 1994; Kim *et al*., 2005). The good or bad effects of cosmetics on teenagers may be from choosing to buy cosmetics that are not quality or the use of cosmetics that have expired for a long time, etc.

Therefore, the aim of this study was to measure the microbial contamination, of non-expired and expired cosmetics products among the popular uses in female students via the evaluation of microbial contamination. The questionnaire for cosmetic product types and uses was made to survey female students. The expired and non-expired cosmetics were used to evaluate microbial contamination.

2. Materials and Methods

2.1 Questionnaire for cosmetic product types and uses

This study was carried out at Mae Fah Luang University. The participants were 100 female students with bachelor's degrees students. The online questionnaire was used as a tool to study an interesting type of cosmetics or uses of cosmetic products by participants. The online questionnaire was divided into 3 parts.

Part 1: General information
Part 2: Marketing factors
Part 3: Factor of products

2.2 Preparation and microbial contamination of cosmetic products

As shown in Figure 1, four popular make-up products without opening to use were foundation (expired and non-expired), brush-on (expired and non-expired), mascara (expired and non-expired), lipstick (expired and non-expired). The criteria to select expired products included the expired products for 1 year. The microbial contamination was determined by cotton swab test (Ismaïl *et al.*, 2013). Products (0.3-0.6 g) were taken individually into a sterile glass bottle. Fresh sterile nutrient broth

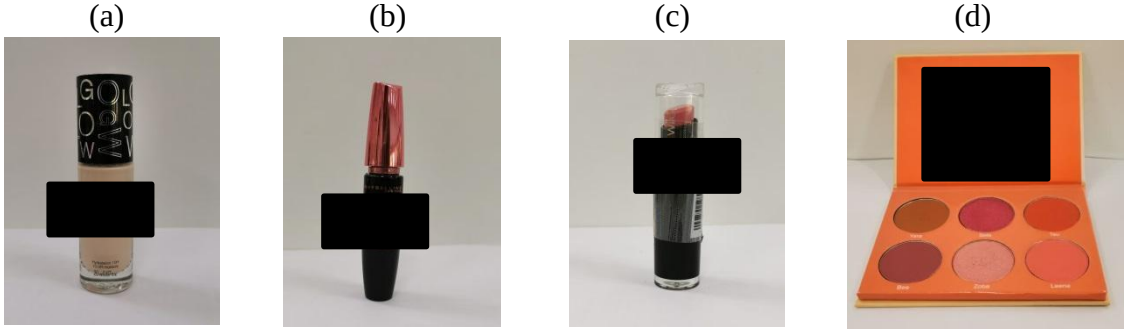

Figure 1 Types of cosmetic products used in the study. (a) Foundation, (b) Mascara, (c) Lipstick, and (d) Brush-on.

(10 ml) was added to each glass bottle and mixed well. Each sample mixture was prepared with a serial of two-fold dilution with the same volume (10 ml) to three concentrations (Table 2). Sterile cotton swabs were soaked into each sample concentration and then spread on plates that have nutrient agars. The experiment in each sample concentration was done in duplicate. All agar plates were incubated at 37 °C for 24 or 48 h. The microbial contamination of each product was measured by the colony count technique (Figure 2).

Table 2 Product concentration prepares by a two-fold dilution technique.

Cosmetic	Product concentrations (mg/ml)		
	1st dilution	2nd dilution	3rd dilution
Expired products			
Foundation	12.50	6.25	3.12
Mascara	3.50	1.75	0.87
Lipstick	13.50	6.62	3.31
Brush-on	14.00	7.00	3.50
Non-expired products			
Foundation	14.25	7.12	3.56
Mascara	10.00	5.00	2.50
Lipstick	16.00	8.00	4.00
Brush-on	12.75	6.37	3.19

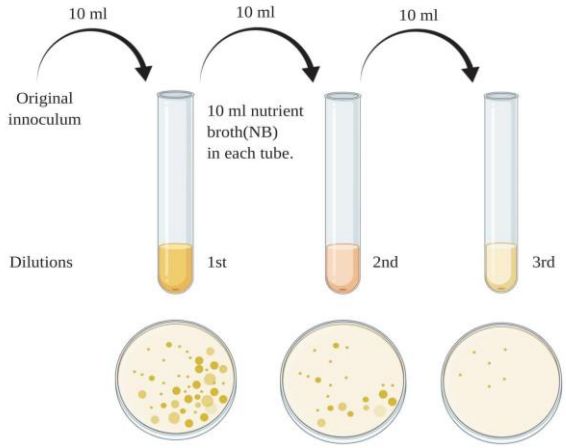

Figure 2 Microbial colony measured by colony count technique**.**

2.3 Gram staining test

The morphology of microbial cells was determined by the Gram-staining test (Sizemore *et al.*, 1990). Briefly, a single colony in agar plate tests was inoculated on a drop of water on the slide and then smeared to be a thin film. The thin film was dried by passing through a flame fire, three times. Drops of crystal violet solution were covered all over a smeared film for 1 minute and then removed with tap water. The iodine solution was dropped on the same film for 1 minute, removed by tap water, and continuously de-stained by 95% ethyl alcohol for 15 seconds. After water washing, the smeared film was stained further by drops of safranin solution for 15 seconds and washed out. The cell morphology of microbes was observed by the microscopic examination technique.

3. Results and Discussion

3.1 Cosmetic product behavior

The questionnaire was created for the study of the types and uses of cosmetic products in female university students. The results are shown in (Table 1) that most of the participants were almost range aged 20-21 years there is an income above 10,000 baht/month. Most of them buy the products online and in beauty stores and are interested in the products of drug store brands from beauty blogger advertising by spending on cosmetic products 1,000-2,500 baht/month. Therefore, these results indicate

those female university students had the frequency to spend on cosmetic products 1,000-2,500 baht/month and their popular types of cosmetic products were make-up.

Table 1 General information, marketing factors, and product factors obtained by an online questionnaire.

Characteristics	**Sample (n=100)**	**Frequencies (rate)**
Age (years)		
18-19	12	0.12
20-21	43	0.43
22-23	42	0.42
24-25	3	0.03
26 or above	0	0
Income (baht/month)		
Below 2,000	4	0.04
2,001-4,000	3	0.03
4,001-6,000	13	0.13
6,001-8,000	18	0.18
8,001-10,000	21	0.21
Above 10,000	41	0.41
How often buying cosmetic products (time/month)		
Never	0	0
1	34	0.34
2	31	0.31
3	13	0.13
More than 3	22	0.22
Place that to buy the products		
Beauty store	43	0.43
Convenience store	7	0.07
Drug store	7	0.07
Online	43	0.43
Brands that buy the most		
Counter brand	47	0.47
Drug store	49	0.49
Luxury brand	4	0.04
Advertising media that prefer to decide to buy a product		
Beauty blogger	66	0.66
Beauty magazine	1	0.01
Billboard	0	0.00
Blog	16	0.16
Social media	4	0.04
TV	0	0.00
Website	13	0.13
How much that spend on cosmetic products (baht/month)		
Less than 1,000	37	0.37
1,000-2,500	40	0.40

2,501-4,000	17	0.17
4,001-5,500	2	0.02
5,5001-7,000	1	0.01
7,001-8,500	0	0.00
8,501-10,000	1	0.01
More than 10,000	2	0.02
Type of cosmetic products that almost buy*		
Cleanser	66	0.66
Day cream	54	0.54
Facial mask	48	0.48
Make up	82	0.82
Night cream	45	0.45
Serum	69	0.69
Sunscreen	69	0.69
Toner	47	0.47

* Participants can select more than one choice.

3.2 Microbial contamination in cosmetic products

Microbial contamination of popular cosmetic products was determined in both expired and non-expired products. The results were shown in (Table 3). The microbial contamination of expired foundation was found at 0-800,000 CFU/g at the first dilution, mascara was found at 1,428.57-2,857.14 CFU/g at the first dilution, 1,142.86-2,285.71 CFU/g at second dilution, 0-1,142.86 CFU/g at third dilution. And lipstick was found 0-339.6 CFU/g at the first dilution. While that of expired brush-on was not found. The microbial contamination of non-expired foundation, mascara, and brush-on was found at 0-210.53 CFU/g, 0-400 CFU/g, and 0-78.43 CFU/g at the first dilution, while that of lipstick was not found. According to the acceptance criteria of the FDA, microbial contamination in cosmetic products must not be over 1,000 CFU/g for the general requirement of product quality and safety (SCCS, 2012; US FDA, 2001). Even though most non-expired products had been contaminated by microbial, the contaminations were less than 1,000 CFU/g. The results indicate that the non-expired products still had quality and safety for use. Most of the expired products, such as foundation, mascara, and lipstick, had been found the microbial contaminations and their contamination levels were over extremely than 1,000 CFU/g. Therefore, the results indicate that the expired products could not be safe for the consumer. The expected possible factors including low quality of raw materials, ingredients, production process, and storage temperature before use could lead to the microbial contamination of cosmetic products, as well as the severity of the contamination.

3.3 Morphology of microbial cells

The results are shown in (Figure 3). Microbial cells in expired cosmetic products, such as foundation, and lipstick were found to be *bacilli* shape and mascara was found to be *bacilli* and *cocci*

Table 3 Microbial contamination in foundation, mascara, lipstick, and brush-on dilution.

Cosmetic	Product concentrations (cfu/g)		
	1st dilution	2nd dilution	3rd dilution
Expired products			
Foundation	0-800,000	0	0
Mascara	1,429-2,857	1,143-2,286	0-1,143
Lipstick	0-340	0	0
Brush-on	0	0	0
Non-expired products			
Foundation	0-211	0	0
Mascara	0-400	0	0
Lipstick	0	0	0
Brush-on	0-79	0	0

shape. Microbial cells in the non-expired cosmetic products, such as foundation, and mascara were found to be *bacilli* shape, while those of non-expired brush-on were found *cocci* shape. The different microbial types found in different products were contaminated by the different causative factors of the production, such as low quality of ingredients, production process, and employer, etc. According to the cosmetic law under the cosmetics act B.E. 2558 (2015) regarding the characterization of cosmetics which prohibits the manufacture, import, or sale. Article 1 cosmetics with the microbiological properties as specified below cosmetic which prohibits production, import, or sale. Cosmetics that detect microorganisms that cause disease are as follows. *Pseudomonas aeruginosa* is Gram-negative, rod-shaped (Wu *et al.*, 2015). *Staphylococcus aureus* is Gram-positive and has a round shape arranged in groups like a bunch of grapes may be a single cell, a pair, or a short string (Abdelbary *et al.*, 2017). *Candida albicans* is a yeast that reproduces by budding (Wiles& Mackenzie, 1987). Regarding the acceptance level, the results of our study suggest that the microbial cells in the cosmetic products may be *Staphylococcus aureus* (Gram-positive *cocci*), and *Pseudomonas aeruginosa* (Gram-negative *bacilli*). Due to from the study under the microscope, it was found that the morphology and Gram of the cells found were similar to those of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

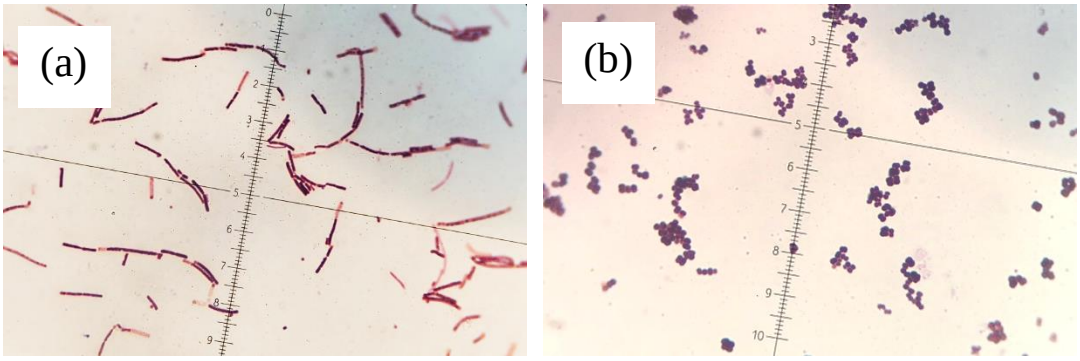

Figure 3 Microbial characteristics in cosmetic products observed under microscope technique. (a) bacilli and (b) cocci at the magnification (1000x).

4. Conclusion

Our results revealed that make-up products were the most popular cosmetic products among female university students. Expired and non-expired make-up products including foundation, mascara, lipstick, and brush-on were used to test for microbial contamination. Microbial contamination was found in most products. Regarding FDA requirements, the microbial contamination must be less than 1,000 CFU/g. Thus, non-expired cosmetic products that were used could be safe for the customer because the microbial contamination was still lower than the FDA standard. However, the microbial contamination of expired cosmetic products was at a severe level, high risk, and unsafe, and could not recommend using because they were extremely contaminated by microbes and over FDA standards.

For further studies, we suggest that the evaluation of microbial contamination should be performed several times and for longer periods. Additionally, that is opened and long-lasting tests for cosmetic products should be investigated and should be further identified for microbial strains.

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The development of cleansing balm containing natural oils

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Abstract

Cleansing balm is oil-based formulation to cleanse any oily dirt. Many facial cleansers contain mineral oil and synthetic surfactants which might cause skin allergy and problems. This study was aimed to develop cleansing balms containing natural oil. Fractionated coconut oil, olive oil, castor oil, sweet almond oil and palm kernel seed oil, as well as cleansers were screened for their cleansing ability against foundation and lipstick. The developed cleansing balm formulations to which the selected natural oils, emollients and emulsifiers used in formulations were then evaluated for cleansing ability, against lipstick and foundation, and satisfactions. The fractionated coconut oil showed the highest ability to remove lipstick and foundation among natural oils. Varying bodying agents for the formulation with suitable texture, spreadability, oiliness and greasiness, for which was further developed the formulations with better cleansing ability. The formulation was adjusted by varying the content of natural oils, and cleansers. All formulations were able to remove foundation, however, incompletely removed for lipstick compared with the benchmark commercial product. The overall satisfaction of the formulation was 3.95±0.62 inferior than commercial product. This study demonstrates the feasibility of natural oils as ingredients in cleansing balm. To improve cleansing ability, lowering amount of natural oil (< 40 %) is recommended for further development.

Keywords: Cleansing ability; Cleansing balm; Makeup remover; Natural oil

1. Introduction

Nowadays, facial makeup products are widely used to promote self-esteem and contributed to physical wellbeing and positive social relationships (Parnsamut *et al*., 2017; Anchieta *et al*., 2021). Most women wear makeup in daily life for the attractiveness and impressment (Tagai *et al*., 2016). Lipstick and foundation are products that are one of most commonly used for makeup applications. Generally, makeup products are waterproof and contain high levels of oil, minerals like titanium dioxide, zinc oxide, iron oxide and kaolin clay, etc. Proper cleaning procedures could cleanse any dirt and make-up product to prevent clogged skin pore and skin problems such as acne which will leave unpreferable signs like dark spots, red spots, acne scars or convex scars. Therefore, makeup removal is important to remove makeup products (Sadeq *et al*., 2019; Li, 2020).

Facial cleanser providing cleanliness with no dryness and removing natural moisturizing factors is preferable. Cleansers are produced to be suitable for various skin types and there are various forms of cleansing products including bar soaps, foams, gels, oil, micellar water, balms, etc. Cleansing balms, generally, are oil-based formulations, thick that appear soft texture like petroleum jelly at room temperature which will be melted into milky form to remove waterproof makeup, sebum and other impurities. They also prevent skin dehydration and improve skin condition (Draelos *et al*., 2018; Hidayah *et al*., 2020). In addition, cleansing balms are not only compatible with the skin to remove emollients but also be easily rinsed off according to emulsifiers in the formulation. In addition, cleansing

products have been used in many services of beauty business, such as clinics and spas. Make-up product removal and facial cleansing are the first priority for the facial treatment, therefore, cleansing balm is alternative in shortening time for this process.

Cleansing balm products are made up from mineral oil that could clog skin pore resulting in skin problems such as irritation, rash, allergic rash and acne inflammation. Besides, synthetic surfactants in the cleansing balm product could also penetrate into the deep layers of the skin causing protein disorder, denaturation, and skin irritation (Novick, 2000; Jurek *et al*., 2019). These concerns also include synthetic emollients which may cause irritation dermatitis, allergic contact dermatitis or allergy (Nola *et al*., 2003; Agner *et al*., 2019). To deal with this problem, natural oils are now focused according to their less toxic and many benefits including moisturizer, antioxidants, antibacterial from various constituents such as essential fatty acids, vitamin A, vitamin C, vitamin B and vitamin E. Natural oils such as coconut oil, olive oil and castor oil have cleansing properties from their compatibility with skin Kim *et al*. (2019), Ngân *et al*. (2021) and Pakkang *et al*. (2018). Therefore, development of cleansing balm containing natural oils is an alternative to reduce the usage of synthetic ingredients. The purpose of this study was to develop cleansing balm formulations containing natural oil as main ingredient and compare their efficiency to remove lipstick and foundation with commercial product.

2. Materials and Methods

2.1 Materials

Caprylyl glycol, ethylhexyl palmitate, fractionated coconut oil, isopropyl palmitate, PEG–20 glyceryl triisostearate and polyethylene were obtained from Chanjao Longevity Co., Ltd., Thailand. Castor oil, olive oil and shea butter were obtained from Nam Sian Co., Ltd., Thailand. Beeswax was obtained from Chemipan Corporation Co., Ltd., Thailand. Cetyl alcohol was obtained from Godrej Industries, Ltd. Polawax was obtained from Sinthai Chemicals & Trading Ltd., Thailand. PEG-7 glyceryl cocoate was obtained from The Real Three (Thailand) Co., Ltd., Thailand. Polysorbate 20 was obtained from NOF Corporation, Japanese. Sorbitan Oleate (Span®80) was obtained from Guangdong Runhua Chemistry Co., Ltd., China. Tocopheryl acetate was obtained from Zhejiang Nhu Company Ltd., China.

2.2 Cleansing ability of natural oils, synthetic emollients and emulsifiers

Natural oils, synthetic emollient and emulsifiers used in this study were tested for their cleansing ability against lipsticks and foundations. Lipstick or foundation (0.02g) was coated on skin with an area of 2.5×2.5 cm. Each sample (0.5 ml) was used to test cleansing ability by rubbing forward and backward (counted as 1 round) for 5 and 10 rounds. The remaining lipstick or foundation removed by different samples were then compared. The suitable natural oils will be then used to develop cleansing balm.

2.3 Development of cleansing balm formulations

The suitable natural oils, synthetic emollients and emulsifiers were selected and being used to formulate cleansing balm by the ratio of fractionated coconut oil and olive oil of 4:1 was selected. The master formulation (F1), adapted from Goggin et al. (2018), contained common selected natural oils as ingredients as shown in Table 1. In brief, ingredients were added in a glass beaker and heated to 70-80°C. The formulation was cooled down to 45-50°C and tocopherol acetate and caprylyl glycol were then added. The master formulation was then adjusted for its texture by varying the ratio of bodying agent and emulsifiers (F1.1-F1.5). Cleansing ability was then developed (F2.1-F2.4) for the comparison with commercial product.

2.4 Cleansing ability

To determine the cleansing ability of cleansing balm, the red lip or foundation (0.02 g) was applied on the arm for a length of 5.5 cm. Then, each cleansing balm (0.5 g) including the commercial product was applied and then rubbed forward and absorbed by tissue paper (counted as 1 round). The process was repeated with no cleansing balm added until the skin was clear. All formulations were compared with commercial product.

Table 1 Ingredient list of cleansing balm formulation.

Ingredients	Function	Cleansing balm (% w/w)								
		F1	F1.1	F1.2	F1.3	F1.4	F1.5	F2.1	F2.2	F2.3	F2.4
Fractionated coconut oil	Cleansing	32	32	32	32	32	32	32	**37**	32	**30**
Olive oil	Cleansing	8	8	8	8	8	8	8	8	8	**5**
Isopropyl palmitate	Cleansing	5	5	5	5	5	5	5	5	5	5
Ethylhexyl palmitate	Cleansing	5	5	5	5	5	5	5	5	**10**	**10**
Polyethylene	Bodying	10	**7.5**	**5**	5	5	5	5	5	5	5
Cetyl alcohol	Bodying	10	**7.5**	**5**	**4**	**3**	**2**	2	2	2	2
Beeswax	Bodying	10	**7.5**	**5**	**4**	**3**	**2**	2	2	2	2
Shea butter	Bodying	10	**7.5**	**5**	5	5	5	5	5	5	5
PEG-7 glyceryl cocoate	Cleansing	7	**13**	**19**	**21**	**23**	**25**	-	**20**	**20**	25
PEG-20 glyceryl triisostearate	Cleansing	-	-	-	-	-	-	**25**	-	-	-
Polysorbate 20	Cleansing	1	**3**	**5**	5	5	5	5	5	5	5
Sorbitan Oleate	Cleansing	1	**3**	**5**	5	5	5	5	5	5	5
Caprylyl Glycol	Preservative	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Tocopherol Acetate	Antioxidant	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

2.4 Preference test

The cleansing balm formulation and benchmark commercial product have been then assessed by double blind test for the preference. 19 participants who have ever used cleansing balm were recruited to the preference test. Lipstick or foundation (0.02 g) was coated on a mirror with an area of 2.5×2.5 cm. Each sample (0.5 g) was tested for their cleansing ability by rubbing forward and backward for 5 cm. The process was repeated with no cleansing balm added until the mirror was clear. After that, they were asked to evaluate their preference including cleansing qualities, texture, spreadability, and oiliness/greasiness. Criteria for the satisfaction level were divided to 5 levels as follows: excellent, good, moderate, low and poor.

2.5 Statistical analysis

The data were compared and analyzed with a independent sample *t* test using IBM SPSS Program version 26.0 (SPSS Inc., Chicago, IL, USA). The preference test values were expressed as the mean ± SD (standard deviation). $P<0.05$ was regarded as statistical difference.

3. Results and Discussion

3.1 Cleansing performance tests of natural oils, synthetic emollients and emulsifiers

Natural oils, synthetic emollients and emulsifiers were tested for their cleansing ability. The cleansing abilities of fractionated coconut oil was more potential than olive oil, almond oil, palm oil and castor oil, respectively, as less intensity of lipstick or foundation observed in the 5^{th} and 10^{th} rounds (Table 2). In the comparison with synthetic emollients (isopropyl palmitate and ethylhexyl palmitate), fractionated coconut oil was similar with slightly different. It was clear that the remaining makeup was similar to synthetic emollients. Fractionated coconut oil used in this study is processed from regular coconut oil by fractionation to obtain high content medium-chain fatty acids (MCFAs) which are caprylic acid (C8) and capric acid (C10) that are more liquid and lighter than regular coconut oil containing lauric acid (C12) as major composition. This fractionated coconut oil, therefore, become light texture and was be easily absorbed into the skin. Olive oil and palm kernel seed oil were similar in lipstick removal, however, olive oil had higher potency in foundation removal. In addition, olive oil is rich in vitamins and antioxidants which could promote skin moisturization and reduce aging effects, especially from sun exposure (Kalogeropoulos *et al*., 2014; Cui *et al*., 2015). Therefore, olive oil was also selected for being used in the formulation by that the ratio of fractionated coconut oil and olive oil was 1:4 according to their potential in removing lipstick and foundation. Synthetic emollients were more potential than emulsifiers. However, all emulsifiers in this study were used for compatibility between water and mixture of synthetic emollient and make-ups.

3.2 Development of cleansing balm

Natural oils, synthetic emollients and emulsifiers were used to develop in the cleansing balm formulation. The master formulation (F1) appeared as a hard-solid characteristic with poor spreadability, therefore, all of the bodying agents (polyethylene, cetyl alcohol, beeswax and shea butter) were reduced. The texture of the adjusted formulations had a trend to be more softened as a dose-dependent decrement of bodying agents. The color of all formulations was milky color and their texture were more easily melted and spread as shown in Table 3. All formulations showed no any sedimentation and phase separation. The F1.5 was the highest spreadability among other formulations according to its lowest spread round. From the results, the F1.5 was then selected to further develop for improving cleansing ability.

3.3 Cleansing ability of cleansing balms

To develop cleansing balm for improving cleansing ability, the F1.5 was selected to formulate the F2.1-F2.4 as can be seen in Table 3. Cleansing ability of the cleansing balm formulations were developed by changing emulsifiers (F2.1), increasing fractionated coconut oil (F2.2), increasing synthesis emollient while decreasing emulsifiers (F2.3) or decreasing fractionated coconut oil (F2.4). All formulations with no any sedimentation and phase separation. From the results, the texture of the formulation F2.4 was lower spreadability than the formulation F2.3, F2.2 and F2.1, respectively. The F2.1 was the best spreadability among the developed formulation due to the decreased bodying agents. The developed cleansing balm formulations (F2.1-F2.4) were tested for their cleansing ability compared with commercial product against lipstick and foundation. Lipstick cleansing ability of F2.1-F2.4 were similar efficiency, however, lower than commercial product as remaining stains observed. Foundation cleansing ability of the F2.1 and F2.3 formulations were more potential than the formulation F2.2 and F2.4. From the results, it could be concluded that the F2.1 formulation was selected for the preference test compared with the commercial product. The commercial product used in this study was milky white color, and had soft texture and easy spreadability, with high efficiency to cleanse lipstick and foundation. The cleansing ability of the F2.1 was inferior than commercial product. This might be due to the high content of natural oil was less compatible with makeup products than synthetic emollients. The reduction of natural oils and increasing other emollients might improve cleansing ability.

Table 2 Cleansing performance tests of natural oils, synthetic oil-based ingredients and emulsifiers on lipstick or foundation.

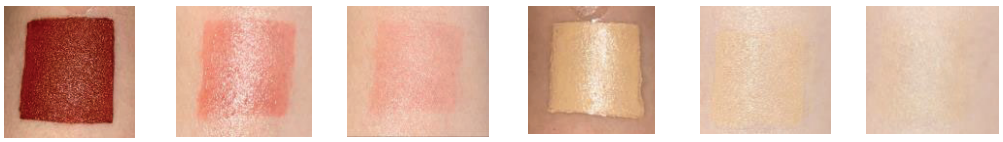
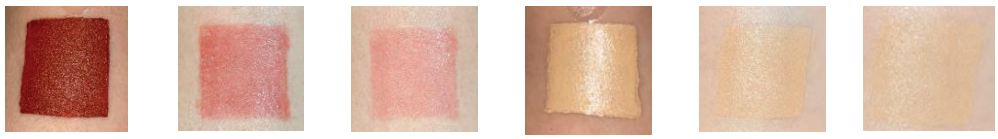
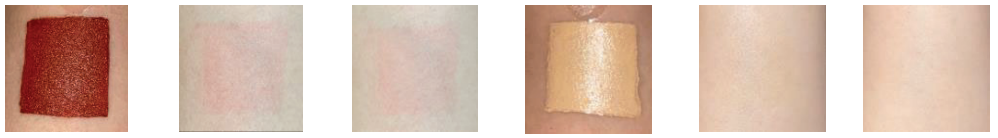
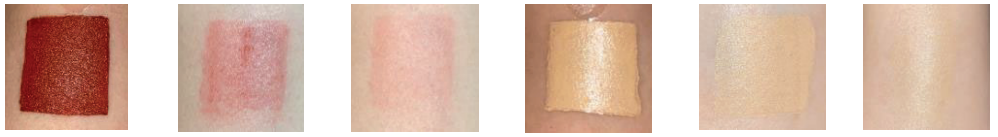
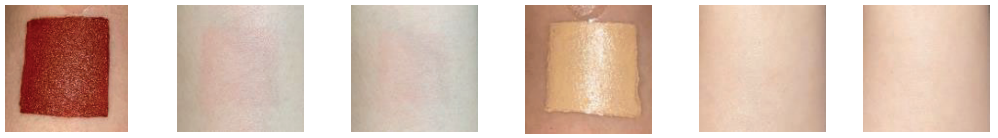
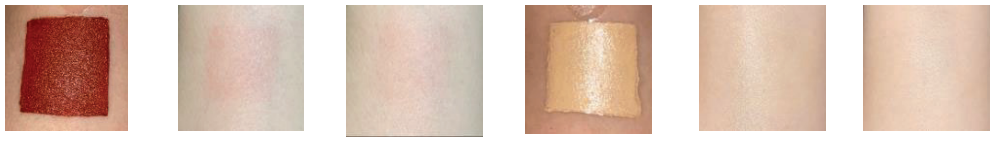
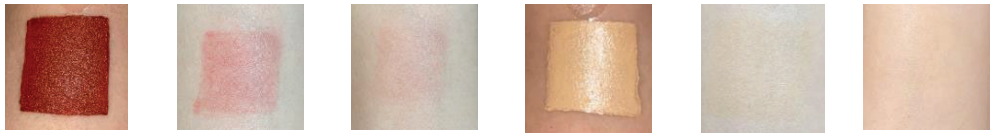
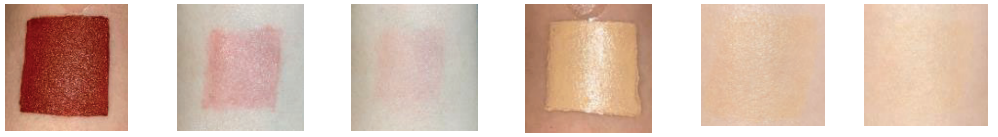
Test samples	Lipstick			Foundation		
	Before	5 rounds	10 rounds	Before	5 rounds	10 rounds
Almond oil						
Olive oil						
Castor oil						
Fractionated coconut oil						
Palm kernel seed oil						
Isopropyl palmitate						
Ethylhexyl palmitate						
PEG-7 Glyceryl cocoate						
PEG-20 glyceryl triisostearate						

Table 2 (Continued)

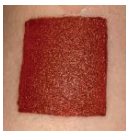
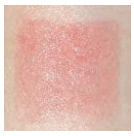
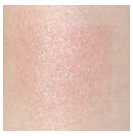
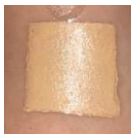
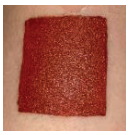
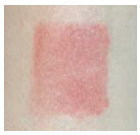
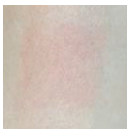
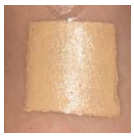
Test samples	Lipstick			Foundation		
	Before	5 rounds	10 rounds	Before	5 rounds	10 rounds
Polysorbate 20						
Sorbitan Oleate 80						

Table 3 Characteristics of cleansing balm.

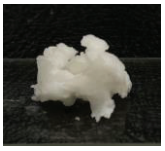
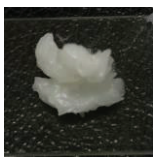
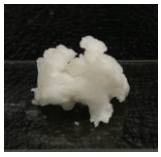
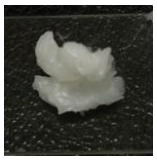
Cleansing balm	Characteristics		Texture adjustment	
	Color	Texture	Spreadability (rounds)	Appearance
F1	Milky white	Hard-solid	> 50	
F1.1	Milky white	Soft-semi solid	29	
F1.2	Milky white	Soft-semi solid	16	
F1.3	Milky white	Soft-semi solid	13	
F1.4	Milky white	Soft-semi solid	16	
F1.5	Milky white	Soft-semi solid	9	

Table 3 (Continued)

Cleansing balm	Characteristics		Texture adjustment	
	Color	Texture	Spreadability (rounds)	Appearance
F1	Milky white	Hard-solid	> 50	
F1.1	Milky white	Soft-semi solid	29	
F1.2	Milky white	Soft-semi solid	16	
F1.3	Milky white	Soft-semi solid	13	
F1.4	Milky white	Soft-semi solid	16	
F1.5	Milky white	Soft-semi solid	9	

3.4 Preference test

For the preference test, 19 participants were blinded from the tested samples. After using the cleansing balm, the results showed that the texture of the developed formulation and the commercial product was similar in terms of softness and easy to spreadability, there was no statistically significant difference. However, the commercial product was more superior in terms of cleaning ability, spreadability and oiliness/greasiness than the developed formulation ($P<0.05$). In an overall rating, commercial product was more preferable as shown in Table 5 ($P<0.05$). The statistical results were consistent with the cleansing ability test in Table 4 that the commercial product was more effective than the formulation F2.1.

4. Conclusion

The present study developed cleansing balm containing natural oils. Different natural oils were screened for their cleansing ability and then selected for cleansing balm formulation development. The fractionated coconut oil was the highest potency among natural oils with similar compared to synthetic

Table 4 Physical characteristic and cleansing ability of cleansing balm formulations.

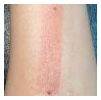
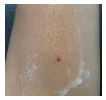
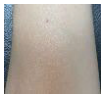
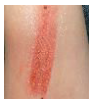
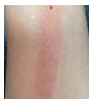
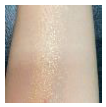
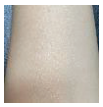
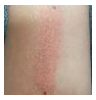
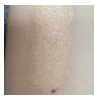
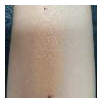
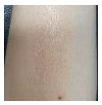
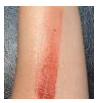
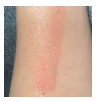
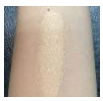
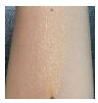
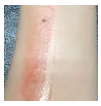
Cleansing balm	Texture		Cleansing adjustment							
	Spreadability (rounds)	Appearance	Rounds		Lipstick			Foundation		
			L	F	Before	3rd rounds	After	Before	3rd rounds	After
*F2.1	8		>10	7						
F2.2	10		>10	8						
F2.3	11		>10	7						
F2.4	12		>10	8						
C	7		>10	7						

Table 5 Preference test of cleansing balm.

Cleansing balm	Cleansing ability	Texture	Spreadability	Oiliness and greasiness	Overall
F2.1	4.00 ± 0.67^{b}	3.74 ± 0.87^{a}	4.05 ± 0.85^{b}	3.95 ± 0.78^{b}	3.95 ± 0.62^{b}
Commercial product	4.84 ± 0.37^{a}	4.26 ± 0.73^{a}	4.74 ± 0.45^{a}	4.74 ± 0.45^{a}	4.58 ± 0.61^{a}

emollient followed by olive oil. The texture of cleansing balm was developed by reducing the bodying agents. The formulations of cleansing balm to improve cleansing ability were developed and the texture was not different compared with commercial product. However, cleansing ability of the developed was inferior than commercial product. This study demonstrated fractionated coconut oil and olive oil could be used.

5. Acknowledgements

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Stability enhancement of ascorbic acid in oil-in-water emulsion

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Abstract

Ascorbic acid is useful in the cosmetic industry because it has antioxidant properties for the skin and as pigment reducing properties. However, ascorbic acid is unstable. It is being destroyed by light, oxygen, high temperatures and alkalinity. The purpose of this study is to determine the stability of ascorbic acid in emulsion formulations. Glycerin, propylene glycol, butylene glycol, and polyethylene glycol-400 were studied as polyols solution added to the formulation, and tocopheryl acetate, olive oil, and ferulic acid were studied as antioxidants of the formulation. The persistence of ascorbic acid at days 7, 14, 21 and 50 was determined by the titration method. It was found that the persistence of ascorbic acid depends on the amount and type of polyols in the formulation. The 50-day ascorbic acid level for glycerin was 91.08 ±0.66%, followed by propylene glycol (87.46±2.05%), polyethylene glycol-400 (87.04±1.17%), and butylene glycol (85.96±1.18%). The effect of antioxidant added to the formula showed that the persistence of ascorbic acid in the formula depended on the type of antioxidant. At 1%w/w antioxidants, tocopheryl acetate gave the highest 50-day residual content of ascorbic acid at 91.86±1.13%, followed by ferulic acid at 90.01±1.33%, and the lowest is olive oil at 83.57±1.28%. In conclusion, it is recommended that adding glycerin and tocopheryl acetate to the system is best to slow down ascorbic acid breakdown in emulsion preparation.

Keywords: Antioxidant; Ascorbic acid; Emulsion; Polyols; Stability

1. Introduction

Ascorbic acid or vitamin C is a water-soluble molecule consisting of six carbon atoms (Sunil Kumar et al., 2017). It is one of the naturally occurring antioxidants (Talakoub et al., 2009) and in most plants and some animals, ascorbic acid can be synthesized from glucose in the body. However, humans and some vertebrates lack of enzyme namely L-glucono-gamma lactone oxidase, which is required for ascorbic acid synthesis in the body, therefore, it is necessary to consume from the outside sources only (Farris, 2009). Ascorbic acid is very useful in the cosmetic industry because it has been discovered to have antioxidant properties for the skin, which results in slower skin degeneration (Telang, 2013). It helps reduce sun-induced skin aging or photoaging (Rhie et al., 2001) and helps to stimulate collagen production of the skin (Nusgens et al., 2001). Ascorbic acid also acts as a pigment reducing agent (depigmenting agent) to inhibit the skin's melanin production as a result of skin whitening effect (Pinnell et al., 2001, Farris, 2005, Telang, 2013). L-ascorbic acid has the physical and chemical properties of a weak organic acid that is soluble in water and unstable, easily oxidized or destroyed by light and oxygen, high temperatures and alkaline conditions (Pielesz et al., 2017). The stability of ascorbic acid has been studied by comparing the amount of ascorbic acid in different solution mediums and studying the effect of pH and solution viscosity on decomposition. The decompose rate of ascorbic

acid was found to be unaffected by viscosity but glycerin and propylene glycol solutions were able to help stabilizing ascorbic acid. Moreover, an alkaline condition was found to accelerate the decomposition of ascorbic acid (Bandelin and Tuschhoff, 1955). Furthermore, studies on the addition of antioxidants, stabilizers, and vitamins to increase the stability of ascorbic acid show that alpha-tocopherol (vitamin E) and stabilizers such as citric acid, tartaric acid and boronic acid has the property to slow down the degradation of ascorbic acid. The mechanism of these agents results from antioxidant properties resulting in slowing down the oxidation process of ascorbic acid (Sheraz et al., 2015). Based on the review information, this work aims to investigate the effects of polyols, i.e., glycerin, propylene glycol, butylene glycol, and polyethylene glycol-400, which are widely used in cosmetic preparations, on the stability of ascorbic acid in the emulsion. The combination of antioxidants (tocopheryl acetate, olive oil, and ferulic acid) with the selected polyol on the stability of ascorbic acid emulsion was also reported.

2. Materials and Methods

2.1 Materials

Ascorbic acid was of Fluka, Germany. Iodine and potassium iodide were purchased from Merck, Germany. Glycerin, propylene glycol, butylene glycol, polyethylene glycol-400, olive oil, ferulic acid, tocopheryl acetate, corn starch, mineral oil, phenoxyethanol and polyacrylamide (and) C13-14 isoparaffin (and) laureth-7 (Sepigel 305) are of cosmetic grade.

2.2 Preparation of ascorbic acid emulsion

Dissolution test of ascorbic acid in various solutions, consisting of glycerin, propylene glycol, butylene glycol and polyethylene glycol-400 was studied. The concentration of ascorbic acid was fixed at 10% w/w and the ratio between solvent and water was adjusted. The mixture was mixed in a test tube and a vortex machine was used to help mixing. The highest ratio of solvent that can dissolve ascorbic acid completely was recorded.

The emulsion of 10% w/w ascorbic acid was prepared by cold process method. The proportions of water and each polyol were adjusted according to the dissolution test, and then mixed by overhead mixer. Sepigel-305 was used as and emulsifier and mineral oil was an oil phase. Stability against phase separation of the emulsion was tested by centrifuge method at 6,000 rpm for 20 minutes.

2.3 Determination of ascorbic acid in emulsion

The ascorbic acid emulsions were filled into a glass jar and wrap with aluminum foil to protect from light. They were stored in a cabinet away from light at ambient temperatures. The ascorbic acid content was analyzed after storage for 7, 14, 21 and 50 days by using the titration method (Satpathy et al, 2021).

3. Results and Discussion

3.1 Preparation of ascorbic acid emulsion

The selected polyols, i.e., glycerin, propylene glycol, butylene glycol and polyethylene glycol-400 (PEG-400) are normally used in various cosmetic preparations to act as humectant. They are considered safe to use and affect the sensory finish of the products and help stabilizing the emulsion preparation. The solubility of ascorbic acid in these polyol solvents was first studied. The concentration of ascorbic acid was fixed at 10 %w/w and the optimum ratio between polyols and water was determined to obtain a completely soluble vitamin C in the system. The results were tabulated in Table 1. The obtained information was then used as a guideline to further preparation of ascorbic acid emulsion in Table 2.

All emulsions appear as creamy white cream with soft texture when apply on the skin. The viscosity of emulsions was in the range of 4000-5000 cPs (spindle#6, 120 rpm, Brookfield viscometer). The emulsion was centrifuged at 6000 rpm for 20 minutes and there was no phase separation observed. The ascorbic acid content in each emulsion was determined by titration method. It showed that ascorbic acid decomposed with the experimental period at 7, 14, 21 and 50 days (Figure 1). After 50 days, the amount of ascorbic acid in base formula, which contains no polyol, was 73.12 ± 1.32%. Formulas with polyols

Table 1 The optimum quantity of polyols and water that completely dissolve 10 %w/w ascorbic acid

Polyol	Quantity (% w/w)	
	Polyol	Water
Glycerin	45	45
Propylene glycol	50	40
Butylene glycol	50	40
PEG-400	45	45

Table 2 Formulas of ascorbic acid emulsion with different polyols

Ingredient	%w/w												
	Base	G1	G2	G3	P1	P2	P3	B1	B2	B3	PE1	PE2	PE3
DI water	77.7	37.5	42.5	47.5	37.5	42.5	47.5	37.5	42.5	47.5	37.5	42.5	47.5
Ascorbic acid	10	10	10	10	10	10	10	10	10	10	10	10	10
Polyols	-	40	35	30	40	35	30	40	35	30	40	35	30
Sepicgel-305	2	2	2	2	2	2	2	2	2	2	2	2	2
Mineral oil	10	10	10	10	10	10	10	10	10	10	10	10	10
Phenoxyethanol	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100	100	100	100	100	100	100	100	100

Note: G=glycerin, P= propylene glycol, B=butylene glycol, PE= PEG-400

showed higher amount of ascorbic acid remain in the emulsion. In the formula containing 40% w/w glycerin(G1), the residual amount of ascorbic acid was the highest at 91.08±0.66%, followed by 40% w/w propylene glycol (P1) at 87.46 ± 2.05%, 40% w/w PEG -400 (PE1) 87.04±1.17 % and 40% w/w butylene glycol (B1) 85.96 ± 1.18%. The remaining ascorbic acid contents in the formulas; G1, P1, B1 and PE1 at 50 days are statistically different ($p < 0.05$). Glycerin had the greatest effect on slowing down the degradation rate of ascorbic acid, followed by propylene glycol, polyethylene glycol-400 and butylene glycol. Glycerin is expected to form the strongest hydrogen bond with ascorbic acid, therefore, the oxidation rate is lower (Zhang, 2007). However, it is not yet known exactly which properties of polyol compounds affect degradation rate of ascorbic acid. The polarity of the polyols is one of the properties expected to be related to the slowing of decomposition. A higher polarity solution is very likely to have more oxidation reaction (Mansoor et al., 2012). However, the polarity properties of each substance, by comparing the dielectric constants at 25 °C, was found to be 42.5, 32.0, 22.0 and 13.6 for glycerin, propylene glycol, butylene glycol and PEG-400, respectively (Wyman, 1933). It showed that glycerin is the most polar solvent but from this experiment, it was found that glycerin was the best to slow down the degradation rate of ascorbic acid. So, the polarity may not be the main factor that affects the ability to slow the degradation of ascorbic acid. However, other studies suggested that this may be because hydrogen bonding between the solvent and ascorbic acid that slows down the oxidation reaction of ascorbic acid (Zhang, 2007; Parr et al., 2002).

3.2 Effect of antioxidant on degradation of ascorbic acid

The formula using glycerin which possessed highest persistence of ascorbic acid was selected to study the effect of different antioxidants, i.e., olive oil, ferulic acid, and tocopheryl acetate. Each antioxidant was studied at 0.5 and 1.0 %w/w in the formula and it was found that at higher amount resulting in higher amount of ascorbic acid in the formula over time, Figure 2. At 1% antioxidant, tocopheryl acetate showed highest persistence of ascorbic acid after 50 days (91.86 ± 1.13%) and followed by ferulic acid at 90.01±1.33% and the least is olive oil at 83.57±1.28%. According to Placzek et al. (2005), antioxidants can slow down the degradation of ascorbic acid by binding to free radicals that occur in the system, this can reduce the rate of oxidation of ascorbic acid. In addition, antioxidants can react with ascorbyl radical which being formed by the oxidative degradation of ascorbic acid and then converted back to ascorbate through redox reactions (Sheraz et al., 2015). The stability of ascorbic acid has been investigated by adding riboflavin, nicotinamide and tocopheryl acerate to the emulsion formulation. It was found that tocopheryl acetate can best slow down the decomposition of ascorbic acid (Ahmad et al., 2012).

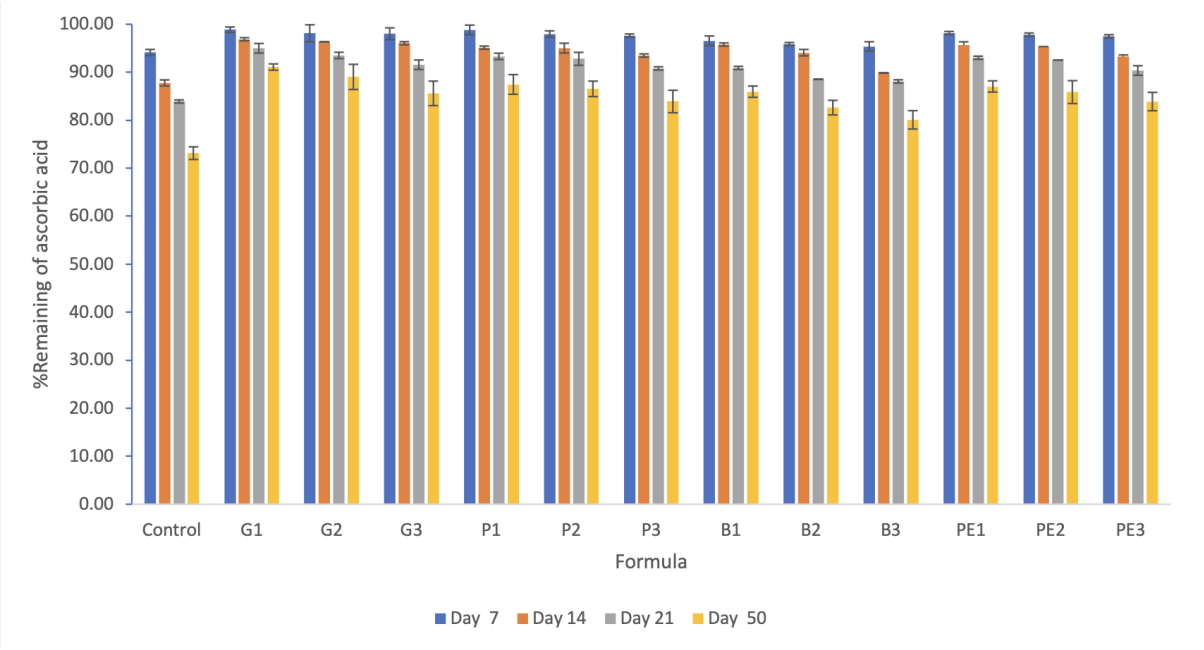

Figure 1 The amount of ascorbic acid in various formulas with polyol solution at 7, 14, 21 and 50 days

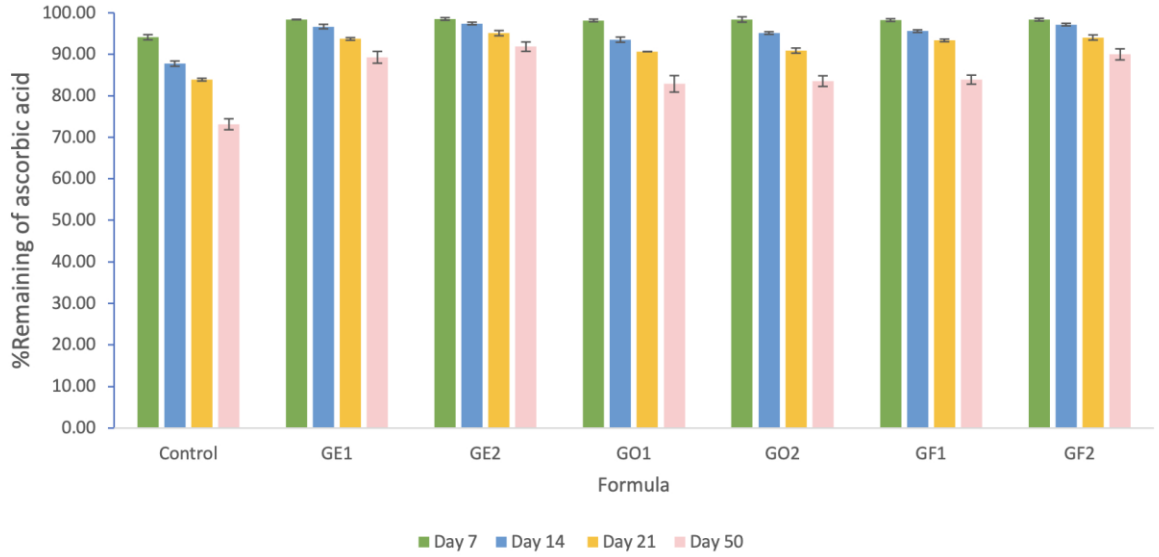

Figure 2 The amount of ascorbic acid in formulas with antioxidant at 7, 14, 21 and 50 days, where GE1 = formula with 0.5% tocopheryl acetate, GE2= formula with 1% tocopheryl acetate, GO1= formula with 0.5% olive oil, GO2= formula with 1% olive oil, GF1= formula with 0.5% ferulic acid, GF2= formula with 1% ferulic acid

4. Conclusion

Glycerin, propylene glycol, polyethylene glycol-400 and butylene glycol were used as co-solubilizing agent for ascorbic acid in emulsion preparation. Different amount and type of polyols can slow down ascorbic acid breakdown and glycerin is best to slow down the breakdown followed by propylene glycol, polyethylene glycol-400 and butylene glycol. Use of tocopheryl acetate, ferulic acid olive oil can also slow down ascorbic acid breakdown due to their antioxidant activity. The combination of glycerin and tocopheryl acetate into emulsion was found to slow down the degradation of ascorbic acid about 18.7% compared with the control system after 50 days at ambient temperature and dark condition. The obtained results are useful for development of a more stable ascorbic acid in emulsion preparation.

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In-vitro antioxidant activity and preliminary encapsulation of carrots (*Daucus caroto*), red cotton tree pollen (*Bombax ceiba*), and roselle calyxes (*Hibiscus sabdariffa*) extracts

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Abstract

This study aimed to evaluate the antioxidant activity and niosome encapsulation of ethanolic and methanolic extracts of purple carrot (PCE and PCM), orange carrot (OCE and OCM), roselle calyxes (RCE and RCM), and red cotton tree pollen (RTE and RTM), respectively. Four selected plants were taken for ethanol and methanol extraction. The antioxidant activity of the extracts was evaluated by DPPH radical scavenging assay. The extract-encapsulated niosomes were prepared by a thin film hydration technique and their particle characterization was performed by scanning electron microscopy (SEM). The yield of crude ethanolic extracts was observed as OCE followed by RCE, PCE, and RTE at 31.39±0.81%, 11.65±0.37%, 11.30±0.47%, and 9.86±0.46%, respectively. The yield of crude methanolic extracts was observed as RCM, OCM, PCM, and RTM at 32.51±0.97%, 31.39±0.81%, 23.11±0.90%, and 10.53±0.24%, respectively. IC_{50} values of the ethanolic extracts were 0.14±0.01 mg/mL in RCE, 0.15±0.01 mg/mL in RTE, and 0.60±0.03 mg/mL in PCE, while those of the methanolic extracts were 0.12±0.01 mg/mL in RCM, 0.15±0.01 mg/mL in RTM, and 0.61±0.06 mg/mL in PCM. OCE and OCM did not show antioxidant activity at even the highest concentration used. The extracts with antioxidant activity were taken further for niosome encapsulation. By SEM observation, the particle characteristics of the extract-encapsulated niosomes were round-like shapes with smooth or rough surfaces and found in a variety of particle sizes observed from nanoscale to microscale. Therefore, all of the studied plants were considered as potential antioxidant sources and, with the preliminary results of niosome development, could be beneficial for the cosmetic industry and beauty technology application.

Keywords: Antioxidant; Carrot; Niosome; Red cotton tree; Roselle

1. Introduction

Oxidative stress is caused by an imbalance between the amount of free radicals in cells and tissues, and the ability of a biological system to eliminate these reactive products. These free radicals are created naturally as a by-product of regular cellular oxygen metabolism. Exogenous causes, such as UV light, stress, and smoking, can also cause them (Katerji *et al.*, 2019). Damage to DNA and macromolecules caused by oxidative stress is related to the initiation and progression of a variety of disorders, including cardiovascular disease, cancer, and aging (Deavall *et al.*, 2012).

Antioxidants are substances that protect cells from oxidative damage and are generated in the human body or obtained from natural sources. Plants are the major sources of natural antioxidants such as herbs, spices, fruits and vegetables. Carrots are a root vegetable and used to produce beverages. Orange carrot (*Daucus carota* L.) contains carotenoids, flavonoids, polyacetylene, vitamins and

minerals. These phytochemicals can help to reduce the risk of many health problems, such as high blood pressure, diabetes, cholesterol, cardiovascular disease, and liver disease (Silva *et.al*., 2014). Purple carrot (*Daucus carota* S.) has a bluish-purple color associated with the presence of large amounts of anthocyanins. It also contains large amounts of carbohydrates, vitamins, and minerals. Therefore, purple carrots are a highly nutritious vegetable (Rasheed *et al*., 2022). Red cotton tree (*Bombax ceiba* L.) is a natural wild tropical tree which is extensively dispersed in Southeast Asian countries. The plant is commonly used in indigenous medicine to treat diabetes and other diseases (Bhargava & Shah, 2020). Anti-inflammatory, anti-oxidant, anti-obesity, hepatoprotective, hypoglycemic, and hypotensive properties have been reported for the plant (Chauhan *et al*., 2018). From a previous study, the ethanol extract from *Bombax ceiba* L. flowers contained a lot of polyphenols and showed a lot of antioxidant activity (Diab *et al*., 2022). The main phytochemicals, such as phenolics, flavonoids, sesquiterpenoids, steroids, naphthoquinones, and neolignans, are found in the plant (Xu *et al*., 2017). Roselle (*Hibiscus sabdariffa* L.) could be used in cuisine and herbal medicine. Bioactive substances from roselle are effective in the treatment of obesity, reducing body weight and suppressing adipogenesis (Ojulari *et al.*, 2019). Extracts of *H. sabdariffa* aerial parts emonstrated antioxidant and antibacterial properties (Jabeura *et al.*, 2017). According to a previous study, roselle calyxes have been found to contain strong antioxidants in several *in vitro* and *in vivo* tests. Phenolic acid, flavonoids, and anthocyanins, all of which can be found in roselle, have been shown to be powerful antioxidants in studies (Singh *et al.*, 2017).

Niosomes are non-ionic surfactant vesicles that can encapsulate both hydrophilic and hydrophobic substances due to their unique structure. The structure of niosomes produced when non-ionic surfactants from the alkyl or dialkyl polyglycerol ether group are combined with cholesterol. They are stable, less harmful, inexpensive, and also do not require particular storage conditions (Khan *et al.*, 2020). Other advantages of niosomes are the better skin penetration, tolerability, enhanced surface adhesion, and sustained release qualities. Due to the advantages, niosomes have been widely studied as a carrier system for active substances (Chen *et al.*, 2019). From previous studies, D-limonene is a monocyclic monoterpene with anti-inflammatory and antioxidant properties and has been studied as a cancer-prevention and therapy component. D-limonene-encapsulated niosomes compared to free D-limonene observed. At a concentration of 20 μM, the niosome formulation demonstrated considerably increased cytotoxic activity against cancer cell lines (Hajizadeh *et al.*, 2019). Rice bran chemical compounds such as oryzanol, ferulic acid, and others have medicinal properties and are used in cosmetic goods. To prevent a rapid degradation, rice bran extracts were encapsulated into niosomes before gel or cream production. The niosomal formulations were experimented in vitro, ex vivo and in vivo and found to be more effective in producing good antioxidant activity and high skin hydration ability (Poorani *et al*., 2020).

Although the active compounds and antioxidant activity of the edible plants including carrots, red cotton tree, and roselle extracts have been reported, studies of antioxidant activities and development of nanoparticles by niosome encapsulation to maintain their properties have not been studied. Therefore, the objective of this study was to evaluate the antioxidant activity of ethanolic and methanolic extracts of carrots, red cotton tree pollen and roselle calyx, as well as their niosome development.

2. Materials and Methods

2.1 Materials

All chemicals such as methanol, ethanol, L-ascorbic acid, 2,2-diphenyl-1-picrylhydrazyl or DPPH, chloroform, Span 60 and cholesterol were obtained from the instrumentation laboratory at the instrumentation laboratory at the School of Cosmetic Science, Mae Fah Luang University. The plant materials consisted of purple carrot, orange carrot, red cotton tree pollens and roselle calyxes. Purple and orange carrots were purchased from Phichit, Thailand. Red cotton tree pollen was bought from Sukhothai, Thailand. Roselle calyxes were bought from Sisaket, Thailand.

2.2 Sample preparation

Purple and orange carrots were washed, peeled and cut into thin slices. The slices were dried by tray-dryer at 55 °C for 24 hours. The red cotton tree pollens were washed and cleaned before being sun-dried for 4-5 days. The roselle calyxes were harvested from fresh flowers and broke the seeds and petals

off the calyxes. Then, the calyxes were washed and sun-dried for 3-5 days, or until completely dry. The dried samples were ground using a blender machine and were sieved using a very fine colander.

2.3 Extraction of purple carrot, orange carrot, red cotton tree pollen, and roselle calyx

For the extraction, 20 g of each powdered sample was mixed with 200 mL of 80% ethanol (ethanol: water, 80:20 v/v) and 80% methanol (methanol: water, 80:20 v/v) by modifying the method from Sultana *et al*. (2009). After that, the mixtures of samples and solvents were stored in the dark for 48-72 hours at room temperature before being filtered through Whatman No.1 filter paper. This procedure was repeated four times. The average of the four repeated samples was used to obtain the final readings, and the standard deviation was calculated for each attempt. To remove the unwanted solvent, the extracted samples for both solvent extractions were evaporated using a rotary evaporator. The extracts were stored and refrigerated until they were analyzed.

2.4 DPPH scavenging activity

DPPH scavenging activity was assessed by modifying the method of Fidrianny *et al.* (2017). Briefly, 0.1 mM methanolic DPPH solution was prepared. The extract samples were prepared in various concentrations. In a 96-well plate, 50 µL of the sample solutions and 200 µL of the DPPH solution were added. After 30 minutes of being left in the dark at room temperature, a microplate reader was used to detect the absorbance at a 517 nm. The standard was ascorbic acid, while the control was DPPH. The antioxidant activity assessment was reported by calculating the average of three determinations analyses of the IC_{50} value and comparing with the ascorbic acid standard.

2.5 Niosome Encapsulation

2.5.1 Preparation of extracts-niosome (extracts encapsulated into niosome).

Niosome encapsulation was prepared by a thin-film hydration technique slightly modified by Theansungnoen *et al*. (2020). In a 50 mL round-bottom flask, a mixture of surfactants (Span 60 148 mg and cholesterol 132 mg) were combined and dissolved in 6 mL of a solvent mixture of ethanol and chloroform (4:2 v/v). The organic solvent was then evaporated, and the thin film was formed using a rotary evaporator under vacuum pressure at 70 °C for 1 hour, followed by 30 minutes of air drying. The resulting film was hydrated with 5 mL of sample extract solutions (5 mg of sample and 5 mL of propylene glycol) by swirling for 15 minutes at 50°C, and the extract-niosomes (red cotton tree and roselle) were gradually produced. For further experiments, the niosome suspension was kept at 4 °C.

2.5.2 Characterization of the morphological surface of niosomes.

The noisome encapsulation was performed by a thin film hydration. Span60 (148 mg) and cholesterol (132 mg) were dissolved to ethanol (4 ml) and chloroform (2 ml). The mixture was added into a round bottom flask. The thin film in the flask was produced using a rotary evaporator at 70°C. and air-dried in the fume hood. Later, samples were measured in the amount of 5 mg and add 5 ml of propylene glycol, then stir until dissolved and poured into an evaporated round bottom flask and swirl in a hot water bath at 50-52 °C until the substance in the flask turns opaque. The niosome sample was distributed on a glass slide by a drop of 10 µL of water onto the glass slides. The niosome samples were then dripped onto glass slides, then air-dried. The sizes and morphological surfaces of niosomes of each sample were evaluated by a scanning electron microscope (SEM) (Theansungnoen *et al*., 2020).

3. Results and Discussion

3.1 The percentage yield of extracts

The extraction yields of ethanolic and methanolic extracts are shown in Table 1. The yield of crude ethanolic extracts was observed as orange carrot ethanolic extract (OCE) followed by roselle calyxes ethanolic extract (RCE), purple carrot ethanolic extract (PCE), and red cotton tree pollen ethanolic extract (RTE) at 31.39±0.81%, 11.65±0.37%, 11.30±0.47%, and 9.86±0.46%, respectively. The yield of crude methanolic extracts was observed as roselle calyxes methanolic extract (RCM), orange carrot methanolic extract (OCM), purple carrot methanolic extract (PCM), and red cotton tree pollen methanolic extract (RTM) at 32.51±0.97%, 31.39±0.81%, 23.11±0.90%, and 10.53±0.24%, respectively. The differentiation in extract yields from the studied plant materials is related to the

Table 1 Extraction yield of ethanolic and methanolic extracts of the selected plants.

Samples	% Yield ± SD
Purple carrot ethanolic extract (PCE)	11.65±0.37
Orange carrot ethanolic extract (OCE)	9.86 ± 0.46
Red cotton tree pollen ethanolic extract (RTE)	11.30±0.47
Roselle calyxes ethanolic extract (RCE)	25.75±0.91
Purple carrot methanolic extract (PCM)	23.11±0.90
Orange carrot methanolic extract (OCM)	31.39±0.81
Red cotton tree pollen methanolic extract (RTM)	10.53±0.24
Roselle calyxes methanolic extract (RCM)	32.51±0.97

different accessibility of extractable components as an effect of diverse phytochemical compositions (Sultana *et al*., 2009). The polarity of the solvent selected affects the extract yield. Furthermore, the yield may be influenced by the compound's solubility and the solvent used. For instance, methanol and ethanol are major polar solvents used to extract some polyphenols, flavonols, alkaloids, and saponin (Jadid *et al*., 2017).

3.2 Antioxidant activities of the selected plant extracts

The antioxidant activity can be assessed by the DPPH scavenging assay. According to antioxidants' ability to transfer electron or hydrogen, the stable free radicals from DPPH react with the antioxidants in the extracts, resulting in a visually observable discoloration from violet to yellow. A lower IC_{50} shows a higher antioxidant activity (Do *et al*., 2014). Table 2 shows the IC_{50} values of both ethanolic and methanolic extracts of the selected plants obtained by the DPPH scavenging activity assay. The IC_{50} values of the ethanolic extracts were 0.14±0.01 mg/mL in RCE, 0.15±0.01 mg/mL in RTE, and 0.60±0.03 mg/mL in PCE, while those of the methanolic extracts were 0.12±0.01 mg/mL in RCM, 0.15±0.01 mg/mL in RTM, and 0.61±0.06 mg/mL in PCM. OCE and OCM did not show antioxidant activity at even the highest concentration used. The quantity of antioxidant components that can be extracted varies depending on the sample. Furthermore, the ability of the extraction solvent to dissolve bioactive compounds might also be critical (Sultana *et al*., 2009).

3.3 The particle characteristics of the extract-encapsulated niosomes

By SEM observation (Figure 1), the particle characteristics of the extract-encapsulated niosomes were round-like shapes with smooth (Figure 1b, 1f and 1g) or rough (Figure 1a, 1c, 1d and 1e). Niosomes are small spherical sacs that can hold various substances within. The structure of the vesicle wall is a double wall which is from the self-arrangement of surfactant molecules containing polar and

Table 2 IC_{50} values of ethanol and methanol extracts of selected plants.

Sample	IC_{50} ± SD (mg/ml)
PCE	0.60 ± 0.03
PCM	0.61 ± 0.06
RTE	0.15 ± 0.01
RTM	0.15 ± 0.01
RCE	0.14 ± 0.01
RCM	0.12 ± 0.01
OCE	*Nd.*
OCM	*Nd.*
L-Ascorbic acid	0.0014 ± 0.00

Nd. means Not detected.

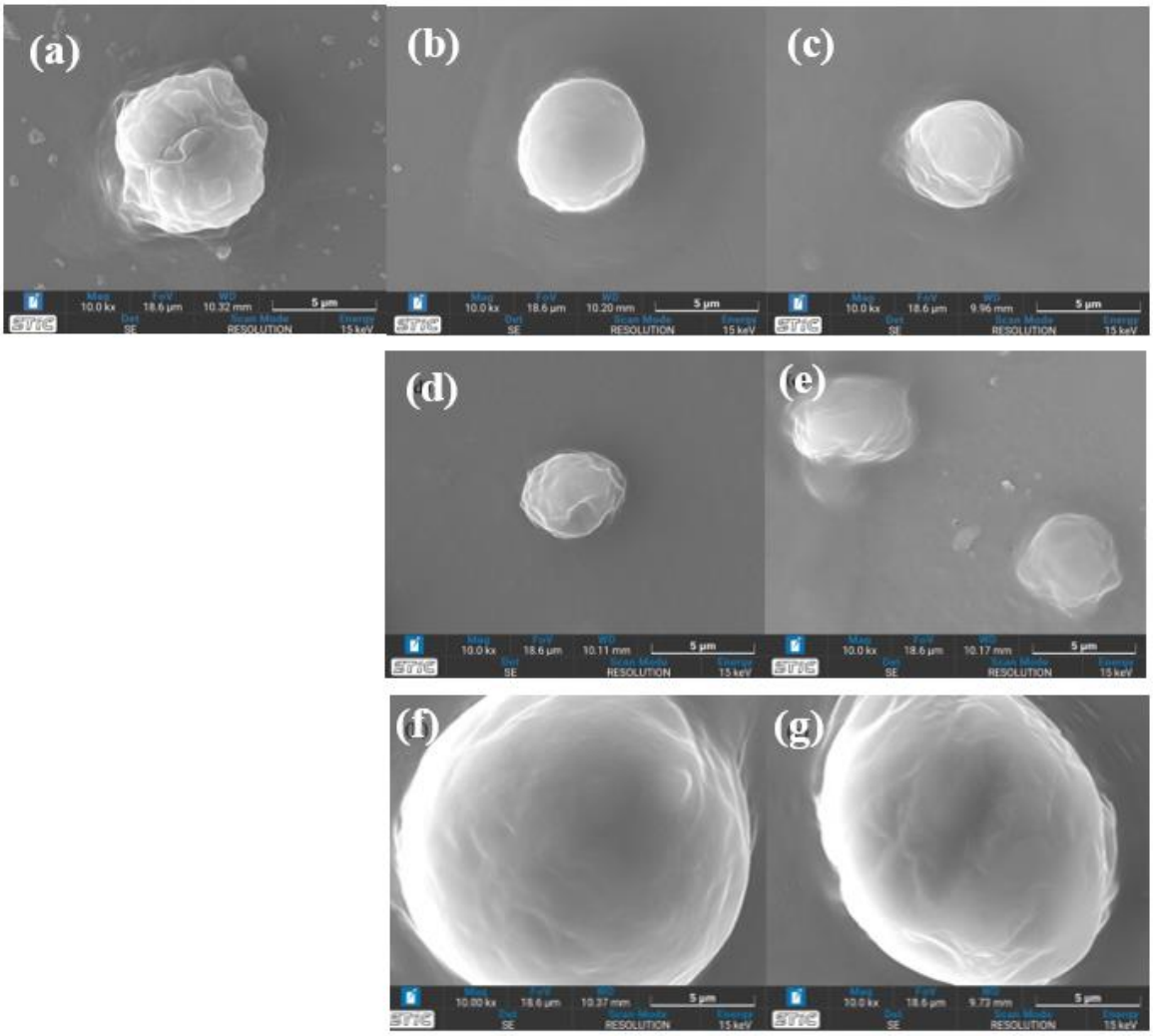

Figure 1 The sizes and morphological surfaces of niosomes with the selected plant extracts. (a) a niosome without extract (Control), (b) a niosome with PCE, (c) a niosome with PCM, (d) a niosome with RTE, (e) a niosome with RTM, (f) a noisome with RCE, and (g) a niosome with RCM.

non-polar properties that exist in the same molecules. They cause arrangement by bringing together parts with the same properties and are stacked as a wall. Therefore, niosomes may be arranged in a single layer or may be produced in more than one class (Thanaketpaisarn *et al.*, 2012).

The diameters of single niosomal vesicle are shown in Table 3. It was found that each niosomal vesicle showed a different diameter mean (4-16 µm). The diameter means of niosome with RTE (14.79 µm) and niosome with RTM (16.42 µm) were wider than the niosome without extract or the control (7.43 µm). Contrastingly, the niosome with PCE had a diameter mean of 6.36 µm, followed by niosome with PCM (5.25 µm), niosome with RCE (4.61 µm), and niosome with RCM (4.06 µm) which were smaller than the control. The physicochemical properties of the encapsulated material can impact the final characteristics of the vesicles (Un *et al*., 2015). Therefore, the results can be assumed that the reduction in the size of both roselle and purple carrot niosomes might be indicative of the interaction between extract and surfactant tending to increase niosomal cohesiveness, resulting in a reduction in size.

4. Conclusion

The ethanolic and methanolic extracts of the selected plants including purple carrot, red cotton tree, and roselle extracts have antioxidant activity as assessed by the DPPH assay. The roselle calyxes extracts (RCE and RCM) and red cotton tree pollen extracts (RTE and RTM) have a greater antioxidant

Table 3 Diameter of the extract-encapsulated niosomes.

Samples	Diameter (µm)
A niosome without extract (Control)	7.43
A noisome with PCE	6.36
A noisome with PCM	5.25
A niosome with RTE	14.79
A niosome with RTM	16.42
A noisome with RCE	4.61
A niosome with RCM	4.06

activity than purple carrot extracts (PCE and PCM) in this study. Niosome encapsulation with the selected plant extracts were successfully performed by a thin-film hydration technique. The niosome morphology has been characterized as spherical shapes with various smooth or rough surfaces, as well as a variety of particle sizes observed from nanoscale to microscale. Therefore, all of the studied plants were considered as antioxidant sources and, with the preliminary results of niosome development, could be beneficial for the cosmetic industry and beauty technology application.

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Mucoadhesive properties of Stingless bee's propolis extract loaded Poloxamer blend sodium carboxymethylcellulose gel

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Abstract

The major compound of stingless bee propolis (SBP) obtained from a mangosteen garden in Chantaburi, Thailand is α-mangostin. It also has antibacterial and antioxidant properties. People in Thailand used SBP to treat mouth ulcers by applying it directly to the ulcer, which does not last long. Controlled and sustained buccal administration has recently become the standard in modern pharmaceutical design in order to improve bioavailability, promote patient compliance, and reduce adverse effects. In situ gel-forming polymeric delivery methods, such as Poloxamer 407 (P407), which has outstanding thermo-sensitive gelling characteristics and biocompatibility, have sparked this interest. However, this gel lacks mucoadhesive characteristics. It will not be able to stay on the buccal long enough for the extracts to be released. Hence the aim of a recent study was to develop a thermosensitive gel by using P407 and sodium carboxymethylcellulose (SCMC) with 0.2% w/w ethanoic SBP extract for buccal cavity. SCMC is mucoadhesive polymers prolong the residence time of the dosage form in the buccal cavity and suitable as matrix material for controlled release. The in-situ gel formulation had produced to assess thermoresponsive behaviour ($T_{Sol\text{-}Gel}$), physical properties, control release, mucoadhesive and antioxidant properties using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. Most formulations display suitable to use for oral application composed of 0.2% w/w SBP in 15% w/w P407/0.05% w/w SCMC. $T_{Sol\text{-}Gel}$ of the formulation was 34.20 ± 0.2°C. The formulation showed a pH value of 5.33 ± 0.02 and the release profile fit to Higuchi model. For the antioxidant property, IC_{50} of the formulation was 79.27 ± 1.69 mg/ml. Furthermore, 0.2% w/w SBP in 15% w/w P407/0.05% w/w SCMC gel showed mucoadhesive properties better than 0.2% w/w SBP in 15% w/w P407 gel without SCMC. This mucoadhesive thermo-sensitive gels with 0.2% w/w SBP extract may have the potential to apply in buccal delivery.

Keywords: Stingless bee's propolis; Poloxamer P407; sodium carboxymethylcellulose; in situ gel

1. Introduction

Five diverse genera of eusocial insects make up the group of stingless bees (Choudhari, Punekar, Ranade, & Paknikar, 2012). These genera are all capable of producing propolis. *Tetragonula pagdeni* Schwarz, *Lepidotrigona ventralis* Smith, and *Lepidotrigona terminata* Smith (Apidae) are commercially grown in Thailand in fruit orchards with manufactured beehives (Umthong, Phuwapraisirisan, Puthong, & Chanchao, 2011). Stingless bees can generate propolis, which is a combination of stingless bee wax, plant exudates, and pollens as sealing glue for their bee hives (S.Jones et al., 2009). A prior study reported on the content and activity of stingless bees' propolis (SBP) obtained

in the same location of a mangosteen orchard in Chantaburi, Thailand. The primary chemical in the SBP was discovered to be α-mangostin. It also had antioxidant properties, antimicrobial properties, and an alpha glucosidase inhibitory effect (Vongsak, Kongkiatpaiboon, Jaisamut, Machana, & Pattarapanich, 2015). SBP was used to cure mouth ulcers in Thailand by putting it straight to the ulcer, which did not persist long. To enhance bioavailability, promote patient compliance, and minimize adverse effects, controlled and sustained buccal delivery has increasingly become the trend in modern pharmaceutical design. This interest has been triggered by in situ gel-forming polymeric delivery methods such as Poloxamer 407 (P407), which has remarkable thermo-sensitive gelling properties and biocompatibility. However, this gel lacks mucoadhesive properties which is low ability to stay on the buccal long enough to release the extracts. The addition of mucoadhesive polymer is a very effective approach for increasing the bioavailability of gel formulations. Many mucoadhesive polymers including sodium carboxymethylcellulose (SCMC), chitosan, and carbopol were examined and SCMC showed extending of the residence period of the dosage form in the buccal cavity. It can be used as a matrix material for controlled release (Priprem et al., 2013; Wu, Chen, & Jin, 2016).

The purpose of this study was to develop a thermosensitive gel containing P407 and SCMC with 0.2% w/w ethanoic SBP extract for the buccal cavity. The physical appearance, $T_{Sol\text{-}Gel}$ temperature, pH, viscosity, and percentage content of SBP extract gel were all investigated. The antioxidant activity of gel was determined in vitro using the (2,2-diphenyl-1-picrylhydrazyl (DPPH) technique. In artificial saliva, the release profile of SBP extract from gel was examined. The viscosity approach was used to investigate the mucoadhesive property.

2. Materials and Methods

2.1 Materials

Stingless bee's propolis was obtained from a farmer at Patthawi, Makham, Chanthaburi, Thailand. Poloxamer 407 (P407), sodium carboxymethylcellulose (SCMC), and mucin from porcine stomach type II were purchased from Sigma Aldrich. All reagents were analytical grade.

2.2 Extraction of SBP

The SBP (4g) was cleaned and split into little pieces individually. The SBP was dried overnight at 50 °C before being macerated in a sonicator bath with ethanol (40 ml) for 60 min at 40 °C. The suspension was centrifuged at 500 g for 20 minutes at 20 °C. The supernatants were collected and evaporated using a rotary evaporator. Then, 400 ml of hexane was used to dewax each extract (Vongsak et al., 2015).

2.2.1 Determination of α-mangostin

The amount of α-mangostin in SBP extracts was analyzed by HPLC. A VertiSep® AQS C18 column (250 mm × 4.6 mm, 5 μm particle size) with a C18 guard column was used. The HPLC analysis was performed according to the method of Pothitirat et al., 2009 with a slightly modification (Pothitirat, Chomnawang, Supabphol, & Gritsanapan, 2009). The elution was performed using gradient solvent systems that consisted of acetonitrile (mobile A) and 0.1% v/v ortho phosphoric acid (mobile B) with a flow rate of 1 mL/min at ambient temperature. The gradient program was as follows: 70% A for 0–15 min, 70% A to 75% A in 3 min, 75% A to 80% A in 1 min, constant at 80% A for 6 min, and 80% A to 70% A in 1 min.?? The wavelength of the UV–visible detector was set at 320 nm.

2.2.2 Determination of anti-oxidant activity

The DPPH technique was used to assess the antioxidant properties of SBP extracts. A 100 L aliquot of 200 M DPPH in ethanol was added to 100 L of extract. The SBP extracts were dissolved in ethanol and then diluted with ethanol to the appropriate concentration. For 30 minutes, the mixture was kept at 37 °C. A microplate analyzer was used to measure the absorbance at 520 nm. The results of the assay were expressed as IC_{50}, which represents the concentration of the extract (μg/mL) required to inhibit 50% of the free radical scavenging activity. The free radical scavenging activity was assessed using equation 1:

$$\% \text{ Inhibition} = [(A_{control}\ 520\ nm - A_{sample}\ 520\ nm)/A_{control}\ 520\ nm] \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

where A_{sample} 520 nm is the absorbance in the presence of the extracts and $A_{control}$ 520 nm is the absorbance of the control. The IC_{50} values were calculated by linear regression of the plots where the

x-axis represented the various concentrations (μg/mL) of the extracts and the y-axis represented the % inhibition.

2.3 Formulation and Characterization of SBP extract gels

The P407 was gently added to the water at 5 °C with mild agitation, and the SCMC was added at various concentrations (0.025, 0.05, and 0.1% w/w). The propolis extract from stingless bees was loaded into the gel at 0.2% w/w. The formulation of in situ gel with and without SBP extract is illustrated in Table 1. Gel (10 g) in a vial containing a magnetic bar was put in a water bath at 15 °C with a thermosensor immersed in the gel and heated with continuous stirring to measure the sol-gel transition temperatures ($T_{sol\text{-}gel}$). $T_{sol\text{-}gel}$ was determined as the temperature measured by the thermistor when the magnetic bar stopped moving leading to gelation.

The visual approach was used to describe the appearance of the SBP extract gels. The pH of the gels was determined using a pH meter (Horiba PH-33, Japan). Rheometer was employed to assess the viscosity of gels (Kinexus Pro, Marvern, model KNX2100, England).

The HPLC technique was used to determine the percentage label quantity of SBP in gel composition. To calculate, the quantity of α-mangostin was taken as a marker. The % label amount of gel was calculated using equation 2:

$$\text{Label amount (\%)} = (La/Lt) \times 100 \text{ ……. (2)}$$

where La is the amount of the SBP extracts that are calculate after formulate and Lt is the initial amount of SBP extracts that are incorporate into formulation.

The In vitro antioxidant activity of SBP extract gels were evaluated using DPPH method. The SBP extract gels were dissolved in ethanol and determined following describe in 2.2.

2.4 Release profile of SBP extract gels

The release profile of SBP extract from the gel was investigated in artificial saliva (Na_2HPO_4 2.38 g, KH_2PO_4 0.19 g and, NaCl 8 g in distilled water 1 L pH 6.8). 10 g of SBP extract gels were immersed in 100 ml of artificial saliva and incubated at 37 °C with 100 rpm shaking. An aliquot of the release medium solution was analyzed by HPLC at a specific time.

2.5 Evaluation of Mucoadhesive Properties SBP extract gels

The in-situ gel's in vitro mucoadhesive strength was determined using the viscosity technique (cylindrical probe 25 ml) (S.Jones et al., 2009). Mucin (6% w/w) was dissolved in artificial saliva. 3 g of mucin solution was combined with 3 g of gels for 15 minutes to prepare the sample. Each formulation's samples were already packed inside the shallow cylindrical probe. Each sample was utilized at 5 g and temperatures of 25 °C, 37 °C, and shear rates of 1, 5, 10, 15, 25, and 25 s^{-1}. These equations were used to examine triplicates of each formulation.

$$\eta_{adhesion} = \eta_{total} - \eta_m - \eta_{gel} \text{ ……(3)}$$

$$\sigma = k\gamma^n \text{……(4)}$$

$$F = \eta_{adhesion}\sigma \text{……...(5)}$$

where $\eta_{adhesion}$ = viscocity of adhesion, η_{total} = viscosity of total (formulation), η_m = viscosity of mucin, η_{gel} = viscosity of gel, γ = shear stress (Pa), σ = shear rate (s^{-1}), k =consistency index, n = dimensionless number and F = mucoadhesive force (N)

3. Results and Discussion

3.1 SBP extraction

Afterwards extraction, the crude extract was collected with the yield of 11.45 ± 1.06 %. The α-mangostin concentration in the SBP extracts was 5.98 ± 0.37 % w/w. SBP extract has an in vitro antioxidant activity (IC_{50}) of 66.96 ± 2.93 μg/ml. Vongsak et al. (2015) found that the IC_{50} of ethanoic SBP extract without wax removal was 122.7 μg/ml in a prior investigation (Vongsak et al., 2015). These

findings suggested that the wax removal SBP extract provided higher antioxidant activity.

3.2 Formulation and characterization

Table 1 shows the formulation of based and SBP gels. The amount of SBP extract used was set at 0.2% w/w, which offered antioxidant capabilities (Vongsak et al., 2015). The appearance of gel based revealed clear colorless and when SBP extract was added, the gel became yellow.

Table 1 Formulation of SBP extract gels

Formulation	P407	0.2 SBP in P407	P407/SCMC	0.2 SBP in P407/ SCMC
SBP (g)	-	0.2	-	0.2
P407 (g)	15	15	15	15
SCMC (g)	-	-	0.05	0.05
Tween 80 (g)	0.75	0.75	0.75	0.75
Sodium Saccharin (g)	0.1	0.1	0.1	0.1
Peppermint oil (g)	0.2	0.2	0.2	0.2
Purified water (g)	q.s. 100	q.s. 100	q.s. 100	q.s. 100

Table 2 displays the $T_{sol\text{-}gel}$, pH value, antioxidant activity (IC_{50}), and content (%) of SBP gels. The sol-gel transition temperatures of all formulations were obtained visually. These findings indicated that the SBP formulation's sol-gel transition temperature reductions may be due to the SBP extract binding to the polyethylene oxide chain in P407. This may enhance polymer dehydration, resulting in neighboring molecule and intermolecular forces that lead to gelling at lower temperatures (S.Jones et al., 2009).

Table 2 Physical and Chemical properties including $T_{sol\text{-}gel}$ Temperature, pH, IC_{50}(mg/ml) and content (%) of of SBP extract gels

Formulation	$T_{sol\text{-}gel}$ (ºC)	pH	IC_{50}(mg/ml)	content (%)
P407	39.17 ± 0.62	5.19 ± 0.02	N/A	N/A
0.2 SBP in P407	34.83 ± 0.29	5.39 ± 0.01	81.1 ± 2.43	99.37 ±1.08
P407/SCMC	39.33 ± 1.25	5.23 ± 0.09	N/A	N/A
0.2 SBP in P407/ SCMC	34.20 ± 0.20	5.33 ± 0.02	79.27 ± 1.69	98.42 ± 2.03

N/A = not available

The pH of gel-based formulations ranged from 5.19 to 5.23. When loaded with SBP extract, the pH of the formulation increased to 5.33-5.39. These findings suggested that the SBP extract in gel formulation had a slight influence on the formulation's pH value. The DPPH radical scavenging activity (IC_{50}) of 0.2 SBP in P407 and P407/ SCMC gel was 81.1 ± 2.43 and 79.27 ± 1.69 mg/ml, respectively. The antioxidant activity of the P407 and P407/SCMC gels was not detected. The remaining percentage of SBP extract in the gel was between 99.37 and 98.42%. This result showed that the SBP extract in the gel formulation did not degrade during the formulation process.

Figure 1 displays the viscosity profile of P407, P407/SCMC, 0.2 SBP in P407, and 0.2 SBP in P407/SCMC gels which had viscosities of 105.71, 104.22, 131.65 and 116.61 mPa·s, respectively, at 25 ºC. The viscosity remained unchanged as the shear rate increase from 1 to 25 S^{-1}. The gel had a Newtonian flow rheology profile at a temperature of 25 °C. Due to a characteristic of poloxamer, the viscosity of the gel significantly increased at 37 ºC. The viscosity of P407, P407/SCMC, 0.2 SBP in P407 and 0.2 SBP in P407/ SCMC gels were 14,766.92, 15,863.41, 14784.61 and 14,798.16 respectively. The viscosity decreased as the shear rate increased from 1 to 25 S^{-1}. At 37 °C, the gel's rheology profile exhibited pseudoplastic flow (Ferreira, Moço, Borghi-Pangoni, Junqueira, &

LucianoBruschi, 2016). These findings suggested that the P407/SCMC containing SBP extract gel had in situ gel properties.

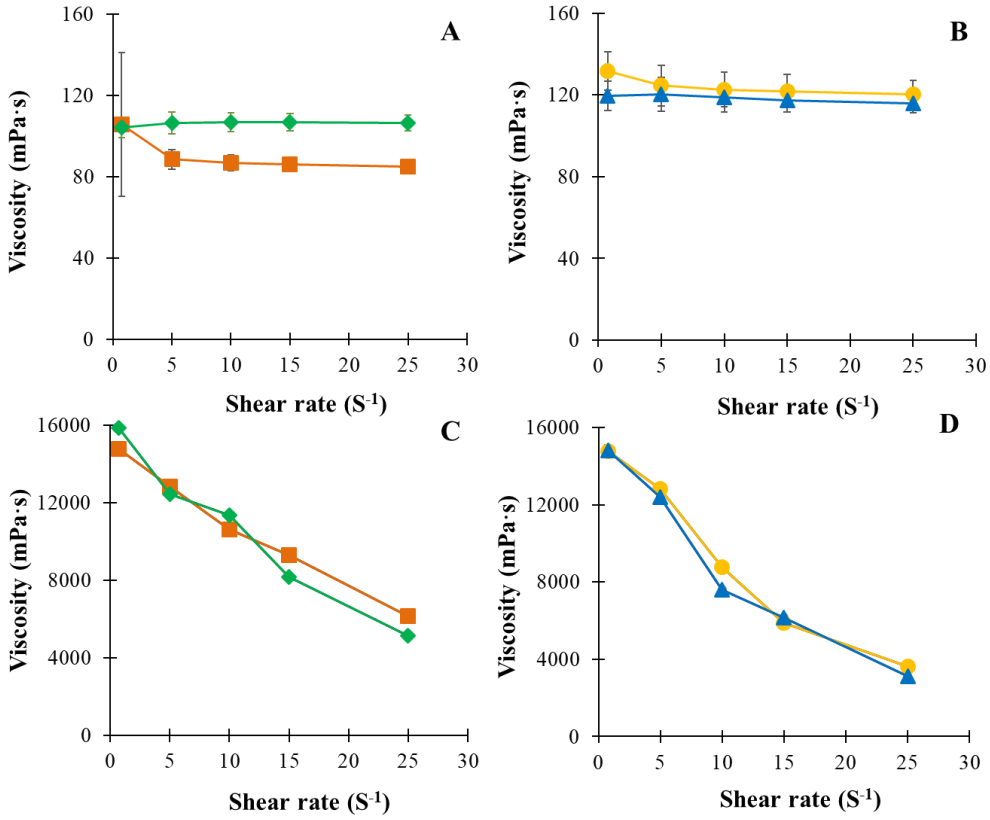

Figure 1 Viscosity profile of P407 gels (■), P407/SCMC gels (◆), 0.2 SBP in P407 gels (●) and 0.2 SBP in P407/ SCMC gels (▲) at 25 °C (A and B) and 37 °C (C and D).

3.3 Release profile of SBP gels

Figure 2 displays the release profile of SBP from gels, determined by HPLC method at 37 °C. The release profile of the 0.2 SBP in P407 and 0.2 SBP in P407/ SCMC gels was not different. The both gels shows slow released (approximately 20 – 30% of extracts release in 12 h). These gels were able to give prolonged action and maintain the therapeutic action for longer periods of time.

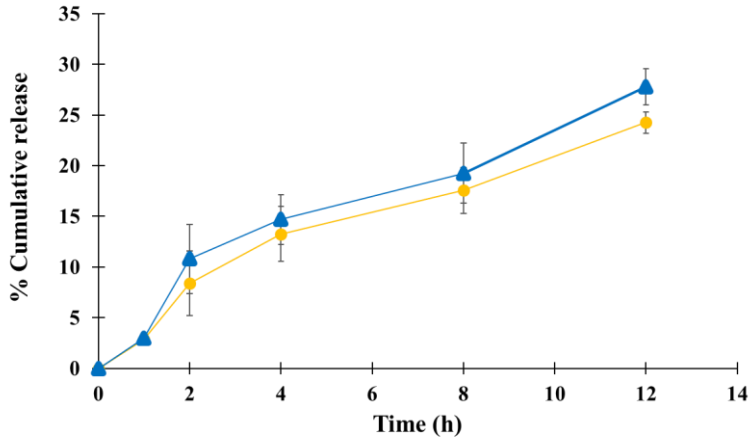

Figure 2 Release character of 0.2 SBP in P407 gels (●) and 0.2 SBP in P407/ SCMC gels (▲).

Table 3 displays the kinetic release of SBP gels. The best fit for each of the models taken into consideration was determined using the regression coefficient (R^2). R^2 values made it obvious that the Higuchi equation plot, which depicts the SBP extract release from the gel as a square root of a time-dependent process based on Fickian diffusion, had the best linearity. Depending on the concentration of SBP extract, diffusion is the process by which SBP extract is moved from the gel matrix into the surrounding in-vitro media. The SBP extract is released and the distance for diffusion grows as the gradient changes. This may explain why the SBP extract diffuses more slowly as the diffusion distance increases (Bansal et al., 2009; Priprem et al., 2013). These results indicated that the Higuchi model described the kinetic release of the 0.2 SBP in P407 and 0.2 SBP in P407/SCMC gels.

Table 3 Kinetic release of SBP extract gels

Formulation	Curve fitting constant (R^2)			
	Zero order	First order	Higuchi	Korsmeyer-peppas
0.2 SBP in P407	0.9458	0.7394	0.9804	0.9332
0.2 SBP in P407/ SCMC	0.9298	0.6932	0.9599	0.8911

3.4 Mucoadhesive Properties of SBP extract gels

Figure 3 displays mucoadhesive properties of the 0.2 SBP in P407 and P407/ SCMC gels. The mucoadhesive forces of 0.2 SBP in P407 and P407/SCMC gels, respectively, were 17.96 and 33.50 at 37 °C. In comparison to 0.2 SBP in P407 gels, the 0.2 SBP in P407/ SCMC gels demonstrated good mucoadhesive properties. Even though the effect of increasing shear rate on viscosity becomes less obvious, the 0.2% w/w SBP in a P407/SCMC formulation remained better viscosity. Mucoadhesion starts when the mucoadhesive material comes direct contact with the tissue. Since high-molecular-weight polymers readily swell in water and expose a large adhesive surface for maximum contact with mucin, the glycoprotein that predominates in the mucous layer, the P407/SCMC formulation exhibits excellent adhesion according to their chemical nature and offers excellent mucoadhesiveness. These results indicated that SCMC could enhance the gel's mucoadhesive properties when added to in situ gel.

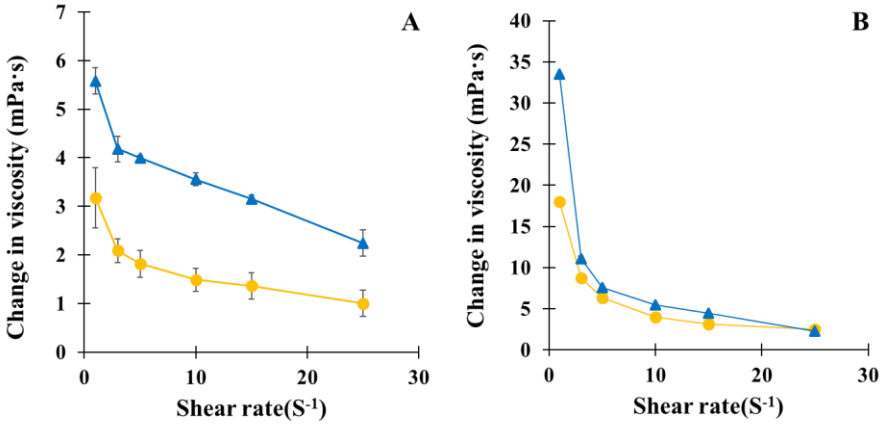

Figure 3 Mucoadhesive properties of 0.2 SBP in P407 gels (●) and 0.2 SBP in P407/ SCMC gels (▲) at 25 °C (A) and 37 °C (B).

4. Conclusion

Formulating the P407/SCMC in-situ gel with SBP extract was accomplished. When loaded with SBP extract, the transparent gels turned yellow. When SBP extract was added, the $T_{sol\text{-}gel}$ temperature and pH values remained constant. The SBP extract in P407 and P407/SCMC gels has antioxidant activity. At temperatures of 25 and 37 °C, the rheology profile of gel was Newtonian flow and

pseudoplastic flow, respectively. The kinetic release of 0.2 SBP in P407 and 0.2 SBP in P407/SCMC gels was compatible with the Higuchi model. The mucoadhesive property of in situ gel may be enhanced by the addition of SCMC. In conclusion, buccal delivery might be a possibility for these mucoadhesive thermo-sensitive gels containing 0.2% w/w SBP extract.

5. Acknowledgements

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Efficacy and safety of the new formulation of herbal coffee on weight control

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Abstract

Overweight and obesity are the majorly underlying factors in public health leading to clinical complications. A new coffee formula with natural extracts was developed to increase metabolic rate and would possibly be used as an alternative for weight control. This study aimed to formulate and study the efficacy, safety, and satisfaction of the twelve herbal coffee formula containing mixture of five herbal extracts, (Guarana (*Paullinia cupana*), Lotus leaves (*Nelumbo nucifera*), Turmeric (*Curcuma loga*), Ginger (*Zingiber officinale*) and Black ginger (*Kaempferia parviflora*). 24 overweight participants were included and randomized into 2 groups, 12 participants for each in which one group received coffee only while another one received the herbal coffee. Once daily consumption for 30 days was assigned in both groups as well as the body composition that was examined on day 0, 14, and 30. The results indicated that the formulation number 5 showed the promising result in stability test and taste preferences. Study results showed the group receiving herbal coffee were statistically different in body fat percentage (0.073 at 90% CI) and serum LDL-cholesterol (0.036 at 95% CI) decreased compared within the group on day 30. The coffee was found to be safe in both groups of participants. The satisfactory level was moderate. In summary, this new herbal coffee tended to be safe with the promising results for weight control in a short-term study. For statistically significant differences of outcomes, further experiment on efficacy and safety in a longer period should be developed.

Keywords: Black ginger; Ginger; Guarana; Herbal coffee; Lotus; Obesity; Overweight; Turmeric

1. Introduction

Overweight and obesity are the excessive or abnormal accumulation of fat or adipose tissue in the body that affects health via its association to the risk of development of cardiovascular disease, cerebrovascular disease, hypertension, and hyperlipidemia. Nowadays, it is a public health problem that is increasing every year (Jitnarin *et al*., 2011; Panuganti *et al*., 2021). The losing weight that negatively affects the body is the use of weight loss drugs, including discourses of degradation, danger and inefficacy (Throsby, 2009).

Natural dietary supplements derived from herbs are another alternative to diet and exercise for weight loss. Researches result on herbal extracts that can reduce weight gain was garcinia (*Garcinia atroviridis*) mixed with caffeine and ginger (*Zingiber officinale*) (Hasani-Ranjbar *et al.*, 2009), ginger (Nazish *et al*., 2016), guarana (*Paullinia cupana*) (Boozer *et al.*, 2001; Lima *et al.*, 2017), lotus (*Nelumbo nucifera*) (Mukherjee *et al*., 2009; Ahn *et al*., 2013; Islam *et al*., 2017), black ginger (*Kaempferia parviflora*) (Yoshino *et al*., 2014; Toda *et al*., 2016), turmeric (*Curcuma loga*) (Sengupta *et al*., 2012; Kim *et al*., 2016; Yadav & Chaudhary, 2016) and coffee (Dellalibera *et al.*, 2006; Onakpoya *et al.*, 2010; Gavrieli *et al.*, 2013; Choi *et al.*, 2016). There are herbal extracts which have been used in

weight control research in humans such as moringa leaves (*Moringa oleifera*), curry leaves (*Muraya koenigii*) and turmeric rhizomes, it was found that the volunteers had a significant reduction in their overall weight and BMI (Sengupta et al., 2012). IQP-GC-101 extract containing garcinia (*Garcinia cambogia*), green tea (*Camellia sinensis*), fresh coffee beans (unroasted *Coffea arabica*) and bananas (*Lagerstroemia speciosa*) can reduce fat percentage, waist circumference and waist to hip ratio (Chong *et al.*, 2014).

Ready-mixed coffee products will be a way to reach coffee consumers who want to take care of their health and weight control. There have been few studies to prove the efficacy of herbal coffee in clinical weight control before. Therefore, the researcher interested in studying the effect of weight control with coffee mixed with five herbal extracts (guarana, lotus leaves, turmeric, ginger and black ginger) to study the efficacy of herbal coffee by analyzing body composition, health data and exercise behavior. In addition, safety studies by assessing hematological biochemical parameters and adverse reactions during research, as well as to assess the satisfaction of consuming products of volunteers.

2. Materials and Methods

2.1 Population and sample

This research is a Quasi-Experimental design (Cook, 2015) which are considered inclusion and exclusion criteria (Table 1). The study was approved by Faculty of Pharmaceutical sciences, Burapha university human ethical committee on June 2018, EC number 21/2018. An overview of the clinical study is provided in Figure 1. Briefly, sample size of this study from Jacob Cohen's tables (Cohen, 1988), the power was 0.8 and the effect size was 0.50 then the sample size was 12 people per group. The participants were randomly distributed using Microsoft Excel. Group 1 who drank one cup of coffee a day (5 g in 100 ml of hot water - placebo) and group 2 who drank one cup of coffee mixed with herbs a day (6.5 g in 100 ml of hot water - treatment).

Table 1 Inclusion and exclusion criteria

Criteria	Details
Inclusion	Adults age 20-45 years.
	BMI 22-39.9 kg/m^2 (Girdhar *et al*., 2016).
	Behavior of drinking 1-2 cups of coffee per day.
	Weight remained stable for the past 3 months before participating in the study.
	Smoking not more than or equal to 50 pack-year.
	No family history that no cardiovascular disease.
	No history of allergy to products from coffee, guarana, ginger, turmeric, black ginger, lotus and the sweetener (Acesulfame potassium).
	Did not donate blood within 3 months before the study.
	Not pregnant or breastfeeding or taking birth control pills.
	No history of disease or received medication to treat non-communicable diseases including cardiovascular disease, diabetes, hypertension, hyperlipidemia, thyroid disease, Cushing's disease or other contagious disease.
	Not take the weight loss drugs or dietary supplements that affect appetite and body metabolism within 3 months before the study.
	Willingness to volunteer to participate in the trial and sign the informed consent form.
Exclusion	Refusing to participate in the study.
	Withdrawing from the study.
	The side effects were estimated that if continued it could be harmful to the participants.

2.2 Data collection

All participants filled a questionnaire, providing details of energy intake and expenditure at the baseline evaluation and during follow up evaluations every weeks. At the baseline and evaluation during follow up at 14 and 30 days, all participants were measured body composition parameters such as body mass index (BMI), percent body fat (PBF), fat free mass (FFM), muscle mass, total body water (TBW), waist circumference (WC), hip circumference (HC), waist-hip-ratio (WHR), arm circumference (AC), right arm fat mass (RAFM) and left arm fat mass (LAFM) using Bioimpedance Analysis (ACCUNIQ, model BC360).

For an assessment of safety, the volunteers had to abstain from food and water for 8 hours and blood samples (8-10 ml) were collected at the baseline and after 30 days to study parameters including cholesterol, HDL cholesterol (HDL-C), LDL cholesterol (LDL-C), triglyceride, glucose, blood urea nitrogen (BUN), serum creatinine, aspartate aminotransferase (AST)/serum glutamic-oxaloacetic transaminase (SGOT), alanine transaminase (ALT)/serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALP). Both groups were required to continue coffee intake daily for 30 days. Follow up for side effects weekly, if during the experiment, participants have anaphylaxis such as rash, hives, swelling of the face, shortness of breath, difficulty breathing and whistling, stop eating immediately and go to the hospital. If symptoms such as fever, cold, diarrhea are present, refer the investigator to assess the risk and record the herbal coffee used in the side effect and adverse drug reaction log book during the trial. The satisfaction of herbal coffee was assessed after 30 days by using questionnaire i.e., color, smell, taste, packaging and overall satisfaction with a satisfaction rating of 1-5, which the score 1 = very dissatisfied, 2 = dissatisfied, 3 = satisfied nor dissatisfied, 4 = satisfied and 5 = very satisfied.

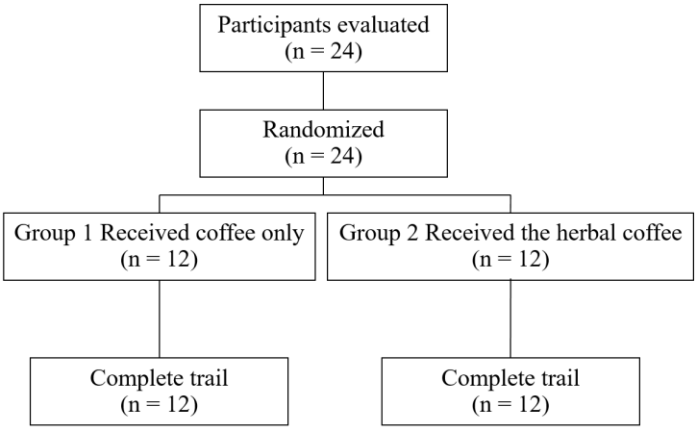

Figure 1 Flow chart of study design. Evaluations of body composition were done at baseline (day 0) and on 14 and 30 days. Assessment of hematology was done at baseline and 30 days. Assessment of satisfaction was done only at after 30 days

2.3 Data analysis

A statistical software package was used to analyze the data and set the confidence interval (CI) at 95% ($p < 0.05$) and 90% ($p < 0.1$) are considered statistically significant and at 90% confidence interval is using to study trends in comparison of each variance). Statistics used to analyze data include Paired t-test, Analysis of covariance (ANCOVA) and Repeated measures ANCOVA. Paired t-test used to compare the efficiency of weight control and safety effects before and after intake coffee in the same group. ANCOVA used to compare the efficiency of weight control between the two groups at 14 and 30 days. Repeated measures ANCOVA used to compare the efficiency of weight control overall between the two groups and used to draw a graph comparing the weight control efficiency. Both ANCOVA and Repeated measures ANCOVA have eating, exercise and before drinking coffee is a covariate factor.

3. Results and Discussion

3.1 Population

The baseline characteristics of 24 participants from Chonburi province were provided in Table 2 that was similar with respect to gender, age and BMI as well as energy intake and expenditure of participants found that the average total energy intake from food (kcal) per day and total energy expenditure from exercise (kcal) per day was not different (Table 3).

Table 2 Baseline characteristics of participants in both study groups (Numbers and percentages)

Baseline variables	Group 1 (n = 12)		Group 2 (n = 12)	
	n	%	n	%
Gender				
Men	7	58	4	33
Women	5	42	8	67
Age (y)				
≥ 20-29	10	84	11	92
≥ 30-39	1	8	1	8
≥ 40-45	1	8	0	0
BMI (kg/m^2)				
≥22-22.9 (Normal range)	1	8	0	0
≥23-24.9 (Overweight)	4	33	4	33
≥25-29.9 (Obese I)	5	42	6	50
≥30-39.9 (Obese II)	2	17	2	17

Table 3 Energy intake and expenditure of participants in both study groups during the trial (Mean values and standard deviations)

Energy intake and expenditure	Group 1 (Mean ± SD)	Group 2 (Mean ± SD)
Energy intake from food (kcal)		
0 week	1,458.20 ± 456.20	1,317.25 ± 416.96
1 week	1,200.53 ± 417.19	1,206.44 ± 511.06
2 weeks	1,164.86 ± 380.01	1,054.55 ± 406.83
3 weeks	1,120.00 ± 352.87	1,044.85 ± 318.30
4 weeks	1,104.59 ± 310.61	1,124.25 ± 426.61
Total average (kcal/day)	1,209.64 ± 56.31	1,149.47 ± 68.52
Energy expended from exercise (kcal)		
0 week	174.69 ± 404.95	34.05 ± 48.41
1 week	149.68 ± 301.26	33.60 ± 45.54
2 weeks	84.76 ± 213.03	71.19 ± 109.84
3 weeks	104.77 ± 257.83	73.33 ± 121.09
4 weeks	94.10 ± 244.53	45.34 ± 78.47
Total average (kcal/day)	121.60 ± 284.32	51.50 ± 34.51

3.2 The efficacy of new formulation of herbal coffee on weight control

The present study evaluated the efficacy of herbal coffee (group 2, G2) on weight loss in comparison with control (group 1, G1) that shows the all parameters of body composition were not significantly different at $p<0.05$ and $p<0.1$ (Table 4). The selection of samples in both groups with similar body composition was a good representation of participant when comparing the baseline differences of each group on 14 and 30 days, there was no statistically significant difference at $p<0.05$ and $p<0.1$ either.

When comparing the mean BMI before and after consuming coffee within the same group, it was found that both group 1 and group 2 had no statistically significant difference at $p<0.05$ and $p<0.1$ in mean BMI within the same group, both at 95% CI and 90% CI ($p = 0.102$ and 0.383, respectively). As

Table 4 Body composition before and after 14 and 30 days in both study groups (Mean values and standard deviations; mean differences (MD))

Body composition	Baseline (Mean ± SD)		p	14 days (MD)		p	30 days (MD)		p
	G1	G2		G1	G2		G1	G2	
BMI (kg/m^2)	26.43 ± 3.35	27.44 ± 4.71	0.548	0.008	0.042	0.873	0.350	-0.417	0.142
PBF (%)	28.63 ± 6.53	32.12 ± 3.74	0.122	-0.017	-0.483	0.215	-0.233	-0.600	0.440
FFM (kg)	53.25 ± 9.26	49.80 ± 9.99	0.390	-0.150	0.408	0.399	0.317	0.542	0.689
Fat mass (kg)	21.71 ± 7.46	23.95 ± 7.19	0.462	-0.017	-0.317	0.229	-0.117	-0.367	0.542
Muscle mass (kg)	29.33 ± 5.16	27.36 ± 5.45	0.373	-0.067	0.308	0.341	0.200	0.217	0.960
TBW (L)	38.33 ± 6.67	35.91 ± 7.15	0.401	-0.092	0.158	0.593	0.242	0.350	0.790
WC (cm.)	89.21 ± 10.11	89.07 ± 13.44	0.976	-0.033	-0.392	0.284	-0.150	-1.508	0.234
HC (cm.)	106.73 ± 7.48	105.05 ± 9.06	0.624	0.229	-1.496	0.177	0.104	-0.714	0.338
WHR	0.83 ± 0.04	0.84 ± 0.06	0.613	-0.002	0.003	0.444	-0.002	0.000	0.836
AC (cm.)	31.63 ± 3.21	30.84 ± 3.70	0.584	-0.317	-0.708	0.594	-0.633	-0.633	0.660
RAFM (kg)	1.35 ± 0.49	1.46± 0.44	0.574	0.011	-0.002	0.528	0.015	-0.007	0.491
LAFM (kg)	1.36 ± 0.48	1.48 ± 0.43	0.520	0.006	-0.024	0.107	0.005	-0.019	0.407

well as comparing the mean BMI between the two groups on days 14 and 30 of coffee intake (p = 0.686 and 0.156, respectively). After 30 days of consuming coffee, the mean BMI of both groups was not significantly different at 95% CI and 90% CI (p = 0.698) but the graph shows that group 2 tended to decrease mean BMI while group 1 tended to increase (Figure 2).

When comparing the mean PBF before and after consuming coffee within the same group, group 1 had no statistically significant difference in mean PBF within the same group despite the 95% CI and 90% CI (p = 0.524) while the second group had a statistically significant decrease in mean body fat percentage within the same group at 90% CI but not statistically significant at 95% CI (p = 0.073). A comparison of mean PBF between the two groups comparing the 14 days, 30 days of coffee intake and after 30 days, found that neither group had a statistically significant difference in mean PBF at 95% CI and 90% CI (p = 0.402, 0.490 and 0.247, respectively). But from the graph, it was found that the second group tended to decrease the average PBF (Figure 3). According to research on the effectiveness of weight control, it was found that the PBF was more likely to decrease when compared within the herbal coffee group, consistent with a 12-week human study with black ginger extract (Yoshino *et al.*, 2014) and a study with guarana extract in volunteers for 8 weeks (Boozer *et al.*, 2001), found a statistically significant reduction in PBF.

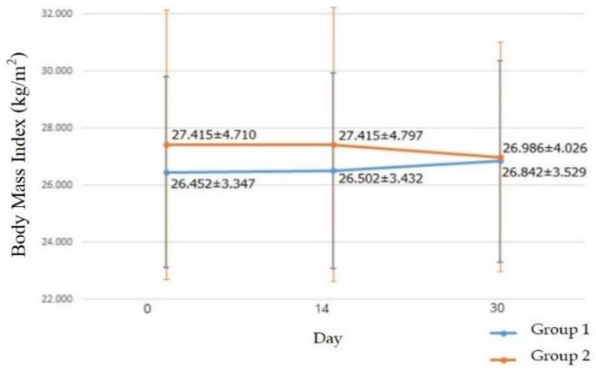

Figure 2 The mean body mass index compared within and between groups (Group 1 is coffee and group 2 is herbal coffee).

A review of the literature describes a possible mechanism for weight control of five herbal coffees (Figure 4). Coffee reduces appetite (Onakpoya *et al.*, 2010; Gavrieli *et al.*, 2013). Guarana seed extract prevents pre-adipose tissue from developing into adipose tissue by increasing anti-adipogenic up-regulation. The genes Wnt10b, Wnt3a, Wnt1, Gata3 and Dlk1 increase the down-regulation of the pro-adipogenic genes Cebpα, Pparγ and Creb1, as well as stimulate the β-catenin nuclear translocation, resulting in decreased adipogenesis (Lima *et al.*, 2017). Black ginger Root Extract stimulates up

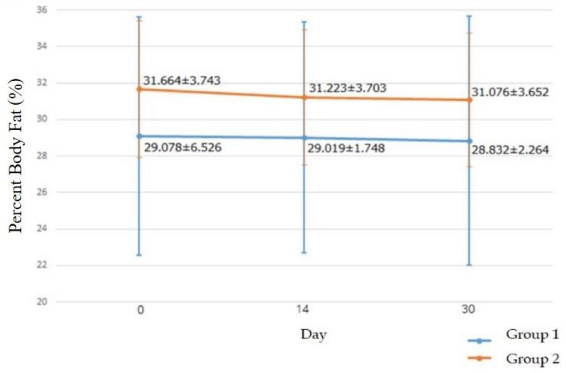

Figure 3 The mean PBF compared within and between groups (Group 1 is coffee and group 2 is herbal coffee).

regulation of UCP1 protein in brown adipose tissue to burn more energy resulting in non-shivering thermogenesis, thus increasing the breakdown of fat (Yoshino *et al*., 2014). Lotus leaf extract inhibits pancreatic lipase and adipocyte differentiation (Ahn *et al.*, 2013; Sharma *et al.*, 2016). Turmeric rhizome extract reduced white adipose tissue by suppressing adipocyte differentiation and lipogenesis (Sengupta *et al.*, 2012). Turmeric extract increases the level of adiponectin fat oxidation fat metabolism (Yamauchi *et al.*, 2002). In research by Dellaliberaet *et al.*, (2006) found a statistically significant mean weight loss after 60 days of coffee bean extract intake due to the fact that coffee is a rich source of chlorogenic acids, chlorogenic acids have both antioxidant, decrease oxidative stress caused by oxidation of lipoproteins in the body, inhibits the activity of Na^+ electrochemical thereby reducing the influx of glucose into cells and inhibit the work of glucose-6-phosphate at the pancreas to balance water use more sugar in the body. The mechanism by which coffee competes with adenosine in binding to receptors that are commonly found in all organs. It was found that coffee stimulates the breakdown of fat more during exercise. When higher doses of coffee, the rate of energy metabolism in the body increases as well (Stern *et al.*, 2013). In addition, Roshan *et al*., (2018) studied the effect of using fresh coffee bean extract capsules at a dosage of 400 mg twice daily with a main meal for 64 days in combination with a diet. It was found that weight, waist circumference and BMI had a downward trend ($p = 0.057$, 0.009 and 0.055, respectively) compared to the control group. When comparing the duration of the reference studies with this study, the duration of the study was considered too short and should be increased to 60 days to see a difference in weight control with 5 types of herbal coffee that is clearer.

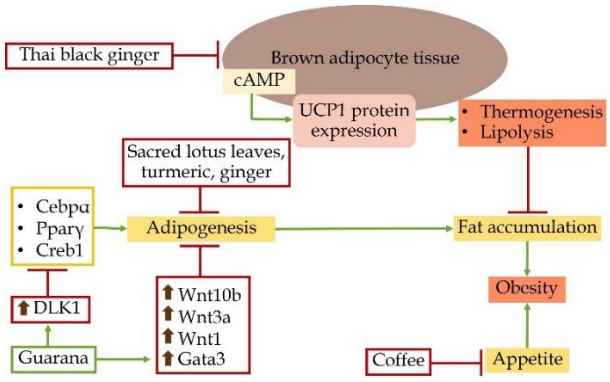

Figure 4 Possible mechanisms of the new herbal coffee on weight control

3.3 The results of safety of new formulation of herbal coffee on weight control

Adverse reactions were found in 2 people of the second group has diarrhea during the first week and 1 person had nausea, representing 17% and 8%, respectively. A daily dose of 1,200 mg of ginger extract significantly reduced gastric emptying time and simulated gastrointestinal peristalsis in healthy volunteers (Wu *et al*., 2008). Coffee was also found to increase colon motility and bowel movements (Sloots *et al.*, 2005). This may explain the cause of diarrhea in the herbal coffee group.

Table 5 The herbal coffee hematological biochemical profiles.

Parameters	Study period	Group 1			Group 2		
		Mean ± SD	MD	P	Mean ± SD	MD	P
Cholesterol (0 -200 mg/dL)	Baseline	211.50 ± 42.86	1.652	0.716	181.00 ± 22.80	6.515	0.212
	After 30 days	213.15 ± 42.08			174.49 ± 19.71		
HDL-C (30 -70 mg/dL)	Baseline	46.33 ± 12.11	1.489	0.429	48.42 ± 7.93	3.488	0.065*
	After 30 days	47.82 ± 10.23			51.91 ± 10.34		
LDL-C (25 -160 mg/dL)	Baseline	142.00 ± 40.230	0.364	0.942	115.25 ± 18.27	8.886	0.036**
	After 30 days	142.36 ± 38.76			106.36 ± 15.82		
Triglyceride (0 -150 mg/dL)	Baseline	115.83 ± 71.50	0.727	0.951	87.33 ± 34.60	5.894	0.381
	After 30 days	115.11 ± 57.07			81.44 ± 34.87		
Glucose (74 -100 mg/dL)	Baseline	90.75 ± 4.58	0.867	0.619	87.42 ± 6.30	1.784	0.186
	After 30 days	91.62 ± 5.80			89.20 ± 5.33		
BUN (7.900 -20.200 mg/dL)	Baseline	11.83 ± 4.35	0.059	0.965	10.48 ± 2.38	0.333	0.630
	After 30 days	11.77 ± 2.09			10.81 ± 1.52		
Serum creatinine (0.840 -1.240 mg/dL)	Baseline	0.82 ± 0.10	0.039	0.055*	0.75 ± 0.17	0.005	0.743
	After 30 days	0.86 ± 0.08			0.75 ± 0.15		
AST/SGOT (0 -35 U/L)	Baseline	20.00 ± 4.59	1.947	0.160	19.50 ± 9.04	1.720	0.082*
	After 30 days	18.05 ± 3.33			21.22 ± 10.01		
ALT/SGPT (0 -45 U/L)	Baseline	26.17 ± 12.55	4.936	0.033**	27.08 ± 17.98	3.231	0.201
	After 30 days	21.23 ± 9.27			30.31 ± 23.03		
ALP (30 -120 U/L)	Baseline	61.412± 14.68	3.633	0.027**	72.17 ± 20.92	5.133	0.078*
	After 30 days	57.78 ± 12.14			67.03 ± 14.58		

*Significantly different at p<0.1 (90% CI)
**Significantly different at p<0.05 (95% CI)

Laboratory tests on safety found that a total of 12 people in group 1 had all of the normal clinical chemistry blood test values both before and after coffee ingestion. Group 2 of 12 people showed statistically significant increases in HDL-C and AST between before and after coffee intake at 90% but not statistically at 95% (p = 0.065 and 0.082, respectively). In addition, there was a statistically significant decrease in LDL-C between before and after herbal coffee intake, both at 95% and 90% CI (p = 0.036). A statistically significant decrease in alkaline phosphatase was found between before and after coffee consumption at 90% CI but not statistically at 95% CI (p = 0.078) in Table 5.

In terms of safety, it was found that the significantly increased HDL and decreased LDL in the herbal coffee group may be related to the activity of lotus leaf extract which is consistent with the research of Islam *et al.*, (2017) found that lotus leaf extract increased HDL levels and decreased LDL-cholesterol levels statistically significant in rat Long-Evans. A study by Yoshino *et al.*, (2018) found that black ginger extract increased the fat burning process in the body, resulting in an increase in volunteers' HDL content. Hepatoprotective effects have been reported of low doses of caffeine (5 and 10 mg/kg) in rats treated with carbon tetrachloride and there was a statistically significant decrease in liver enzyme values including alkaline phosphatase (Sloots *et al.*, 2005). This may be the source of a statistically significant decrease in the alkaline phosphatase of the volunteers in both groups. In addition, the herbal coffee group received turmeric extract containing curcumin which has been reported to significantly reduce liver injury (Hasani-Ranjbar *et al.*, 2009). The increased AST value in the herbal coffee group may be attributed to black ginger that has been reported to significantly alter liver enzyme levels (Sudwan *et al.*, 2006).

3.4 The results of a satisfaction study of new formulation of herbal coffee on weight control

The satisfaction containing color, smell, taste, packaging and overall satisfaction of the herbal coffee are shown in satisfied nor dissatisfied scores (Table 6)**.**

4. Conclusion

The herbal coffee showed the tendency to increase HLD-C and reduce LDL-C and body fat percentage which would be applied to control weight when continuously used and longer duration as well as the safety reported the blood biochemistry profiles were in normal range. Further improving the satisfaction in terms of taste and scent are important to be commercialized.

Table 6 The satisfaction of herbal coffee.

Satisfaction	Score (Mean ± SD)	Mean
Color	3.00 ± 1.00	Satisfied nor dissatisfied
Smell	3.00 ± 0.87	Satisfied nor dissatisfied
Taste	3.00 ± 1.04	Satisfied nor dissatisfied
Packaging	3.00 ± 1.03	Satisfied nor dissatisfied
Overall satisfaction	3.00 ± 0.95	Satisfied nor dissatisfied

(The satisfaction of herbal coffee was assessed after 30 days by using questionnaire i.e., color, smell, taste, packaging and overall satisfaction with a satisfaction rating of 1-5, which the score 1 = very dissatisfied, 2 = dissatisfied, 3 = satisfied nor dissatisfied, 4 = satisfied and 5 = very satisfied.)

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6. References

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Preparation of Tamarind Ready to Drink Powder by Spray Dried Technique

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Abstract

Tamarind (*Tamarindus indica* L.*)* is a popular Thai fruit enriched with vitamin C. This study aimed to produce instant powder of tamarind for "ready to drink" product, suitable with modern life style. Maltodextrin (food grade, DE 10-12) was selected to use as a spray dried carrier in this study. Ripe tamarind mass was boiled and mixed with 3 different concentrations of maltodextrin (10, 20 and 30%W/V). Liquid mixtures were taken into spray dried process. Powder products from spray dried process were tested for the physicochemical, chemical and biological properties. The result revealed that all three products have yellowish color. The moisture content percentage of the products obtained from 3 maltodextrin concentrations range from 3.40 to 5.41. Total phenolic contents were determined by Follin ciocalteu method with gallic acid (GA) as a standard. The total phenolic contents of the products were between 0.42-1.08 mg GAE/g powder. The antioxidant activity was evaluated by ABTS method using vitamin C and Trolox as standards. The antioxidant capacity of the products expressed in Vitamin C equivalent antioxidant capacity (VCEAC) ranges from 415.10 to 670.75 µg VCEAC /g powder. The antioxidant capacity expressed in Trolox equivalence antioxidant capacity (TEAC) ranged from 462.60 to 753.15 µg TEAC /g powder. Result from reconstitution time test shows that the three powder products are completely dissolved within 30-99 sec giving good taste solutions. It could be concluded that these three-prototype spray dried tamarind powders have good physicochemical characteristic, good taste and fast dissolved. The three products show antioxidant activity proved by detectable phenolic compound which are beneficial for our health.

Keywords: Powder for instant drink; Tamarind; Spray dried powder; *Tamarindus indica* L., Antioxidant

1. Introduction

Cost of nutritional supplement drink in Thailand market is now continuously growing. In 2021 the total market value was 9,654 million baht. In 2022, it is expected to grow by 5-7% to reach 10 billion baht as mention in Thairath money economy report (Techasiriprapa N.2022). People in 2020s pay more attention on their health. Hectic life style may result in high consumption of fast food and lack of nutrient in long term. "Ready to drink powder" for instant drink is one of product type that suitable for this need. Tamarind (*Tamarindus indica L.)* is a popular Thai fruit. Hundred grams of tamarind give 240 kcal. Apart from carbohydrates fats and proteins, tamarind contains vitamin A, B1, B2, B3, B5, B6, B9, choline, vitamin C, vitamin

E, vitamin K and several minerals such as calcium, copper, iron, magnesium, phosphorus, potassium, selenium, sodium and zinc (https://en.wikipedia.org/wiki/Tamarind). Tamarind is being used in many Thai dishes and also popular as freshly prepared refreshment drink. Thus, this study aimed to prepare tamarind spray dried powder for instant drink. The ratio of carrier used in spray dry process was studied. The physicochemical properties, chemicals content and bioactivity of final products were evaluated.

2. Materials and Methods

2.1 Materials

Fresh and fully ripened tamarind pods were procured from the local market (Thailand) and stored in refrigerator until needed for the experiment. Maltodextrin (food grade, DE 10-12) was used as a carrier agent. Folin ciocalteu (FC) and Gallic acid (Sigma) as reagent and standard for determine total phenolic contents and $ABTS^{+}$ (2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid)) and Ascorbic acid (vitamin C) as reagent and standard for radical scavenging assay were used in the experiment.

2.2 Sample Preparation and Spray Drying

After the outer hard shell and seeds of tamarind fruits were removed manually, tamarind fruits were soaked and boiled in water. The mixture was blended in a laboratory type blender and filtered to separate fiber and other residues. Tamarind and maltodextrin were mixed in the ratio 1:10, 1:20, 1:30 and spray dried at inlet air temperatures of 190 °C. After drying, powders were recovered from the cyclone and cylindrical parts of dryer chamber and then weighed to determine powder recovery, packed in polyethylene bags and stored in desiccator containing silica gel till further analysis. The powders were analyzed for color, particle size analysis, moisture content, flow ability, bulk and tapped density, dissolution time, pH, amount of total phenolic content and antioxidative activity.

2.3 Particle Morphology and Particle Size Analysis.

Particle morphology and particles size analysis was carried out by using light microscope with image analysis program. The particle size of powders was expressed as the average of particle diameter (n=200).

2.4 Moisture Content

The moisture content of tamarind pulp powders was determined according to loss on drying (LOD) method in United States Pharmacopeia (USP). Tamarind spray dried powder was weighed before and after drying in hot air oven at 105 °C for 2 h. The samples were analyzed and expressed as %Loss on drying in triplicate and the mean was recorded.

2.5 Flowability

Flowability of tamarind pulp powders was measured by fixed funnel method. After tamarind spray dried powder 40 g passing funnel, the radius and height were measured and calculated as angle of repose. Flow ability was characterized using angle of repose data following powder flow USP. The samples were analyzed in triplicate and the mean was recorded.

2.6 Bulk and Tapped Density

The bulk density was measured according to the method followed by USP. Accurately weighed 40 g of the powder was freely poured into a 100 mL graduated glass cylinder. The samples were then tapped by lifting and dropping the cylinder using digital tapped density apparatus 300 rpm for 2 min. The bulk and tapped density were calculated as ratio of mass of the powder to the volume occupied in the cylinder. The measurements were carried out in triplicate for all the powder samples. In addition, Hausner index was calculated and determined flow ability following <1174> powder flow USP. The samples were analyzed in triplicate and the mean was recorded.

2.7 Dissolution Test

One gram of the sample was weighed and placed in 50 mL of distilled water, followed by mixing using magnetic stirrer with temperature control at 8, 30 and 80 °C. The time taken by powder sample

to fully dissolved was recorded using an electronic timer. The samples were analyzed in triplicate and the mean was recorded.

2.8 pH of reconstituted solution of tamarind spray dried powder

Tamarind spray dried powder 1 g in 50 mL of distilled water was mixed and determined pH using pH meter. The samples were analyzed in triplicate and the mean was recorded.

2.9 Determination of total phenolic contents (TPC)

Total phenolic contents (TPCs) of the spray dried products were determined by Folin ciocalteu (FC)'s method using gallic acid as a standard (Rahman N.F.A. *et al*., 2018). The gallic acid was dissolved in methanol and diluted to the concentrations ranged from 0.05 to 0.15 mg/ml. The folin ciocalteu (FC)'s reagent was diluted with distilled water to a 1/10 concentration. To 200 µl of gallic acid solution, 1500 µl of the diluted FC reagent was added. The mixture was mixed by using vortex and let stand at room temperature for 5 minutes before adding 1500 µl of 6% Sodium carbonate (Na_2CO_3). The reaction was mixed well and let stand at room temperature for 90 min. The absorbance was read against blank at 725 nm. The standard calibration curve of gallic acid was created (y=6.552x+0.0364, R^2 = 0.9950).

The tamarind spray dried products were dissolved in distilled water to a concentration of 0.1 g/ml. The TPCs of the sample solutions was determined as described in the standard gallic acid's procedure. The experiments were performed in three replicates. The averaged TPC value were expressed in milligram gallic acid equivalent per g powder of the products (mgGAE/g powder).

2.10 $ABTS^+$- radical scavenging assay

Total antioxidant capacity of the tamarind spray dried products was investigated by $ABTS^+$ (2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid)) radical scavenging assay using vitamin C and Trolox as a standard as previously described in Shahinuzzaman M. *et al*., 2020. The $ABTS^+$ radical was prepared by mixing equal volumes of 7 mM ABTS with 2.45 mM potassium persulfate ($K_2S_2O_8$) solution. The mixture was placed in the darkness at room temperature for 16 h. The $ABTS^+$ radical solution was later diluted with distilled water and adjusted to the 0.7 ± 0.02 absorbance at 734 nm. The standard vitamin C was dissolved in distilled water and diluted to the concentrations ranged from 60 to 160 µg/ml. The standard Trolox was dissolved in methanol and diluted to the concentrations ranged from 50 to 175 µg/ml. The standard solution of 0.1 ml was mixed with 3.9 ml of $ABTS^+$ radical solution and kept in the darkness for 30 min. The absorbance was read at 734 nm against blank. The percentage of inhibition was calculated using the following equation.

$$\%\text{Inhibition} = \frac{(\text{Acontrol} - \text{Asample})}{\text{Acontrol}} \times 100$$

Where Acontrol = absorbance of $ABTS^+$ radical solution

Asample = absorbance of the tested solution

The calibration curve was created from %Inhibition (Y) and concentrations (X) with linear correlation formula y = 0.5658x +1.7747, R^2 = 0.9973.

The tamarind spray dried product was dissolved in distilled water to 0.2 g/ml concentration and tested for antioxidant activity follow the procedure as described in standard vitamin C. The % inhibition of the spray dried product was calculated. The antioxidant capacity of the products was expressed in µg vitamin C equivalent antioxidant capacity per g products (µg VCEAC/ g) and µg Trolox equivalence antioxidant capacity (TEAC) per g products (µg TEAC/ g).

3. Results and discussion

3.1 Powder Recovery and particle size

Powder recovery of tamarind pulp powders produced from spray drying with different ratio of tamarind and maltodextrin is shown in Table 1. The maximum of powder recovery was shown in ratio 1:30 (19.845% powder yield). Powder recovery was increase in high content of maltodextrin. The

obtained spray dried tamarind powder is irregular shape particle with light yellowish color and its particle size was around 35.89-75.97 μm. It was also found that particle size and the more lightness of yellow color of spray dried powder was increased by the higher content of maltodextrin.

Table 1 Powder Recovery and particle size of spray dried tamarind powder

Ratio	Particle size (μm)	Powder Recovery (%Yield)
1:10	35.87	13.428
1:20	46.77	15.456
1:30	75.97	19.845

3.2 Powder characterization and Physicochemical Properties of spray dried powder

Spray dried tamarind pulp powders are characterized and presented in moisture content, bulk and tapped density and flow ability as shown in Table 2. It was found that the highest value of moisture content is 5.46% in the ratio 1:10. The moisture content of the powders is well below the maximal range of 5%, for spray dried products. However, the moisture content of spray dried powder in the ratio 1:20 and 1:30 show 4.35% and 3.29% respectively. The obtained spray dried powder shows lower moisture content when using the higher content of maltodextrin. It was possible that the higher content of maltodextrin could decease water adsorption from the atmosphere. It has been reported about the ability of maltodextrin to forming a moisture-protective barrier after water absorption on the surface of hygroscopic particle. This function could improve the quality of dehydrate product. (Phanindrakumar *et al*., 2005, Gabas et al., 2007, Tong et al., 2008).

The particle properties (density and flowability) are important for processing, packaging, storage, and shipping. Bulk and tapped density of the obtained spray dried powder are around 0.5-0.6 and 0.7-0.8 g/mL respectively. The large amounts of high density of the powder could be easier packed into smaller container. High temperature drying processes are achieved implying a lower shrinkage of the droplets, and so a lower density of the powder (Chegini G.R., Ghobadian B., 2005). It has been reported that increase in inlet air temperature also showed a significant negative effect on the powder bulk density (Khalid M., Pradyuman K., 2016). Maltodextrin may cause an increase in the volume of air trapped in the particles, corresponding to a decrease in the apparent density of the particles (Athanasia M.G., Konstantinos G.A., 2008). From the results of angle of repose and Hausner index, flow ability of spray dried tamarind powder is very poor following the scale of flow ability in USP. However, the flow ability of spray dried powder is greater in the ratio 1:30. The flow ability is better in the higher content of maltodextrin and lower moisture content.

Table 2 Powder characterization of spray dried tamarind powder

Ratio	Angle of repose (°)	Bulk density (g/mL)	Tapped density (g/mL)	Hausner ratio	%LOD
1:10	97	0.50	0.73	1.45	5.46
1:20	63	0.57	0.81	1.39	4.35
1:30	60	0.57	0.79	1.37	3.29

3.3 Dissolution time and pH of reconstituted spray dried tamarind solution

The dissolution test of powder measures its reconstitution speed in water. The results (Table 3) showed that powder produced from the ratio 1:30 takes only 20-38 sec to fully reconstitute in water. All samples (the ratio 1:10, 1:20 and 1:30) showed the short period time to fully reconstituted in water at room temperature (30 °C) around 30-99 sec. The higher temperature could decrease the dissolution time of spray dried tamarind powder. The shorten dissolution period time may be related to the moisture content of the powders or solubility of spray dried powder produced from natural tamarind substance and maltodextrin. Quek et al. (2007) also reported a positive correlation between moisture content and dissolution for spray dried watermelon powders. After reconstitution of tamarind powder in distilled water, the pH value was determined. The lowest of pH solution was shown in the 1:10. It was possible that the higher content of tamarind extract affected to decrease pH and corresponding to greater sour

taste (Da Conceicao Neta E.R. et al., 2007) by organoleptic test of the reconstituted solution when comparing to 1:20 and 1:30 respectively.

Table 3 Dissolution time and pH of spray dried tamarind powder

Ratio	pH	Dissolution time (min:sec)		
		8°C	30°C	80°C
1:10	4.02	1:14	1:39	0:59
1:20	4.12	1:03	0:59	0:55
1:30	4.18	0:38	0:30	0:20

3.4 Total phenolic content, antioxidative activity against $ABTS^+$ radicals of spray dried tamarind powder

The total phenolic contents of the spray dried tamarind products ranged from 0.42-1.08 mg GAE/g powder (Table 4). It was found that the products with lower percentage of carrier contained higher TPCs. The TPCs of the products with 10, 20 and 30% w/v of maltodextrin were 1.08, 0.78 and 0.42 mg GAE/g powder respectively. All the products exhibited antioxidant activity against $ABTS^+$ radicals (compared with Trolox and Vitamin C) with the total antioxidant activity from 462.60 to 753.15 µg TEAC/g powder and 415.10 to 670.75 µg VCEAC /g powder. The product with lower percentage of carrier exhibited higher antioxidant capacity. The total antioxidant capacity of the products with 10, 20 and 30% w/v of maltodextrin were 753.15, 633.11 and 462.60 µg VCEAC/g powder respectively when compared with Trolox. The total antioxidant capacity of the products with 10, 20 and 30% w/v of maltodextrin were 670.75, 564.40 and 415.10 µg VCEAC/g powder respectively when compared with vitamin C. The antioxidant activity is highly correlated to the total phenolic contents of the products (R2 =0.9977-0.9980). The antioxidant activity of tamarind pulp has previously reported. The aqueous and alcohol extracts of ripe tamarind pulps showed DPPH radical scavenging activity and suppressing thiobarbituric acid reactive substances (TBARS) formation (Ugwuona F.U. and Onweluzo J.C., 2013). Aungkuldee W. et al., 2017 has reported the antioxidant activity of tamarind water prepared in the ratio of 1:1 to 1:5 (w:w). The less diluted tamarind-water at 1:1 showed the highest ABTS radical scavenging activity with highest total phenolic contents (0.59 mg GAE/100 g of tamarind-water). Bhusari S.N. and Kumar P. (2014) described the antioxidant activity of tamarind pulp spray dried powder using different carrier agents including maltodextrin (40, 50, 60%), gum Arabic (40, 50, 60%) and whey protein concentrate (10, 20, 30%). The TPCs of the products were ranged from 59.45-131.33 mg of GAE / 100g, which are lower than in our study. This could be resulted from dilution factor of the carrier agent.

Table 4 Total phenolic content, antioxidative activity against $ABTS^+$ radicals of spray dried tamarind powder

Ratio	Total Phenolic content	Antioxidative activity against $ABTS^+$ radicals	
	mg GAE/g powder	(Trolox equivalent antioxidant capacity, µg TEAC/g powder)	(Vitamin C equivalent antioxidant capacity , µg VCEAC /g powder)
1:10	1.08	753.15	670.75
1:20	0.78	633.11	564.40
1:30	0.42	462.60	415.10

4. Conclusion

It could be concluded that these three-prototype spray dried tamarind powders that we prepared have good physicochemical characteristic, good taste and fast dissolved. The three products show antioxidative activity proved by detectable phenolic compound which are beneficial for our health. The amount of maltodextrin used in preparation affect to moisture content, total phenolic compound and antioxidative property of products.

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Possibility of wellness business after Covid-19 pandemic

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Abstract

The objectives of this study were to survey the wellness services experience of Thai people and the marketing mix factors affecting respondents' decisions on wellness services after the Covid-19 pandemic. The results obtained from this research might be used to develop the business model of wellness and is a guideline for entrepreneurs and interested people in the business of wellness after the outbreak of Covid-19 has ended. The questionnaire was created by using Google forms and distributed online from July 2021 to August 2021. There were 1,816 respondents who completed the questionnaire. Most respondents were women aged between 18-25 years. Most of the places to receive wellness services were beauty salons, beauty clinics, gym fitness, and spas. The purposes of receiving wellness services were to look more beautiful, handsome, attractive, and exemplary figures or lose weight. After the Covid-19 pandemic, most respondents want to receive wellness services again, and the place certified by the Amazing Thailand Safety & Health Administration (SHA) is the first important factor that they were concerned about. The business should be provided with high-quality, professional performance, cleanliness, and hygiene. Moreover, the convenience of transportation and technology to support service is needed to be considered when setting up wellness services after Covid-19.

Keywords: Covid-19; Wellness; Wellness business; Wellness services experience; Wellness tourism

1. Introduction

Wellness is focusing on holistic and natural approaches, self-healing, and preventive care. The Global Wellness Institute defined wellness as "the active pursuit of activities, choices, and lifestyles that lead to a state of holistic health". They further defined wellness tourism as "travel associated with the goal of maintaining or enhancing one's personal well-being and includes the pursuit of physical, mental, spiritual or environmental 'wellness' while traveling for either leisure or business (Global Wellness Institute, 2018). Wellness tourism is included in illness prevention tourism, which is categorized into health services and fitness services. Wellness tourism has become a trend in the world community to achieve health and health prevention and gain self-satisfaction furthermore, respondents of wellness tourism are not limited to foreign tourists but have become a lifestyle, especially a consumer community urban in the country (Kaufmann, 2007). Wellness Tourism includes (1) Natural healing assets, (2) Indigenous healing traditions, (3) Medical services, (4) Nature, and (5) Spiritual traditions. Whereas those included in use existing assets are (1) Leisure and recreation spas, (2) Medical/ therapeutic hotel/ clinic spas, (3) Medical/ surgical clinics or hospitals, (4) Medical wellness centers or spas, (5) Holistic retreats, and (6) hotel and resort spas. At the global and regional levels wellness tourism is spread almost evenly in several regions such as Europe, America, and Asia (Widarini *et al*., 2020).

Thailand's main wellness tourism offers are based on spa resorts with the Tourism Authority of Thailand (TAT) promoting 41 spa and wellness resorts in the country. These resorts are especially known for offering traditional Thai massage, which is influenced by traditional medicine and is promoted by TAT for relieving physical and mental tension, as well as reducing migraines, sprains, bruises, anxiety, and improving flexibility (Wayne & Russell, 2020).

Covid-19 is a new pandemic that first erupted in December 2019 in China and spreading rapidly across the world through human-to-human transmission. Most countries all over the world are instituting short-term travel restrictions to stop the spread of infection which increases the concern caused by the Covid-19 pandemic on the tourism industry worldwide (Mertens *et al.,* 2020). The United Nations reports that the recent circumstance of the tourism sector is very worse due to the pandemic. This crisis expanded in the world and the Covid-19 pandemic easily immobilized international tourists' emotional stability (World Tourism Organization., 2020). The impact on the wellness tourism sector was not yet known, but since flights throughout the region have been severely curtailed, this sector and every other tourism sector have been negatively impacted. One can only hope that the virus will soon stop spreading; that a vaccine is discovered and distributed rapidly; and that the tourism industry, including wellness tourism, can resume its growth trajectory.

The objectives of this study were to survey the wellness services experience of respondents and the marketing mix factors affecting respondents' decisions on wellness services after Covid-19. The results obtained from this research might be used to develop the business model of wellness and is a guideline for entrepreneurs and interested people in the business of wellness after the outbreak of Covid-19 has ended.

2. Materials and Methods

2.1 Respondents

The study population was composed of 1,816 respondents who lived in Thailand. The respondents were aged range between 18 to more than 65 years. The inclusion criteria consisted of respondents who agree to join this study by signing the consent questionnaire on Google forms, they use the internet, and can read Thai. The exclusion criteria consisted of people who did not agree to answer the questionnaire. This study was approved by Ethics in Human Research Committee of MFU (No. EC 21099-17).

2.2 Survey design

The questionnaire was created by Google forms (https://forms.gle/fBc4pguZnqsZWTYe9) and distributed the link online via many channels such as Facebook, Instagram, Twitter and e-mail from July 2021 to August 2021. The questionnaire was divided into 3 parts consisting of

Part 1: Personal information such as gender, age, marital status, education, career, and monthly income.

Part 2: Wellness service experiences such as the place to receive wellness services, frequency of receiving wellness services, wellness programs that respondents have been receiving, purposes of choosing wellness services, prices for wellness programs per time, and period of time the respondents receive the wellness service. The confidence level of respondents to receive wellness services; before Covid-19, during Covid-19, and after Covid-19 such as the desire to receive wellness services, feeling safe about receiving the services, and the burden of receiving services.

The confidence level of respondents to receive wellness services was calculated using the Five Point Likert scale. Criteria for interpretation of the importance level were divided into 5 levels as follows: 4.21-5.00 (Very High), 3.41-4.20 (High), 2.61-3.40 (Medium), 1.81-2.60 (Low), and 1.00-1.80 (Very Low).

Part 3: The marketing mix factors affecting respondents' decision on wellness services after Covid-19 including the 7Ps marketing mix consisting of product, price, place, promotion, people, process, and physical evidence.

The marketing mix factors affecting respondents' decision on wellness services after Covid-19 were calculated using the Five Point Likert scale. Criteria for interpretation of the importance level were divided into 5 levels as follows: 4.21-5.00 (Very High), 3.41-4.20 (High), 2.61-3.40 (Medium), 1.81-2.60 (Low), and 1.00-1.80 (Very Low).

2.3 Data analysis

Descriptive statistics were used to describe demographic characteristics and wellness services experience. The results were presented as a frequency, percentage, and Mean ± S.D.

3. Results and Discussion

3.1 Demographic characteristics

The demographic characteristics of the respondents were presented in Table 1. There were 1816 respondents who responded to the online questionnaire. About half of them were women (54.79%). Most respondents aged between 18 to 25 years (57.60%). Most occupations of the respondents were students (55.62%) and most of them are Bachelor's degrees (76.60%). The rate income of respondents was less than 18,000 Baht (40.14%).

Table 1 The general information of respondents (N=1816)

Characteristics	Frequency	Percentage (%)
Gender		
Female	995	54.79
Male	741	40.80
Other	80	4.41
Age (years old)		
18-25	1046	57.60
26-33	310	17.07
34-41	279	15.36
42-49	77	4.24
50-57	46	2.53
58-65	39	2.15
More than 65	19	1.05
Marital status		
Married	417	22.96
Divorced/separated/widowed	234	12.89
Single / never married	1165	64.15
Education		
Below high school	77	4.24
High school	18	0.99
Bachelor's degree	1391	76.60
Higher than bachelor's degree	330	18.17
Occupation		
Student	1010	55.62
Private company employees	338	18.61
Civil servant/state enterprise employee	219	12.06
Business owner/personal business	162	8.92
Others	87	4.79
Income (monthly)		
<18,000 baht	729	40.14
18,001-25,000 baht	603	33.21
25,001-32,000 baht	73	4.02
32,001-39,000 baht	133	7.32
>39,000 baht	278	15.31

3.2 Wellness services experience

The experience of the respondents in receiving wellness services was collected. The result is shown in Table 2. The vast majority of respondents had received health services (95.81%) and just the least of the respondents have never been receiving the service (4.19%). Beauty salons (79.25%), beauty clinics (69.77%), gym/fitness (67.59%), and spas (58.85%) were the most popular locations to receive wellness

services. More than half of them visited their wellness services between one and two times a month (71.95%). The most popular programs were beauty services in clinics (74.14%), and beauty services in salons (72.82%). More than half of them wanted to improve their appearance (79.37%), and lose weight (70.80%). Because people tend to make favorable comparisons to their own past selves (Wilson & Ross, 2000), thus they want to be better than before.

Table 2 Wellness services experience

Respondent behavior	Frequency	Percentage (%)
Wellness services experience		
Ever	1740	95.81
Never	76	4.19
A place to receive wellness service (>1 answer)		
Beauty salon	1379	79.25
Beauty clinic	1214	69.77
Gym/Fitness	1176	67.59
Spa	1024	58.85
Wellness center	627	36.03
Hotel/resorts	416	23.91
Hospital	338	19.43
Frequency of receiving wellness services		
1 - 2 times per month	1252	71.95
3 - 6 times per month	422	24.25
More than 6 months at a time	66	3.80
Wellness programs that respondents have been serving (>1 answer)		
Beauty services in clinic	1290	74.14
Beauty services in salon	1267	72.82
Massage in spa	820	47.13
Facial treatment	738	42.41
Body treatment	683	39.25
Exercise and diet	682	39.20
Aromatherapy	574	32.99
Weight loss or weight control	194	11.15
Herbal healing	178	10.23
Acupuncture	175	10.06
Yoga	97	5.57
Ayurveda	64	3.68
Music therapy	59	3.39
Art therapy	46	2.64
Meditation retreat	34	1.95
Other	2	0.11
The purpose of choosing a wellness program (>1 answer)		
Improve appearance	1381	79.37
Lose weight	1232	70.80
Healthy	667	38.33
Improve self-confidence	660	37.93
Relaxation	596	34.25
Anti-aging	385	22.13
Prevent disease	198	11.38
Balanced body	194	11.15
Elimination of toxins	176	10.11
Try something new	81	4.66

Table 2 Wellness services experience (Cont.)

Respondent behavior	Frequency	Percentage (%)
Price of wellness service		
<1,000 Baht	255	14.66
1,001 - 2,000 Baht	513	29.48
2,001 - 3,000 Baht	420	24.14
3,001 - 4,000 Baht	197	11.32
4,001 - 5,000 Baht	160	9.20
>5,000 Baht	195	11.21
Frequency of receiving wellness service		
08.00 - 16.00	871	50.06
16.01 - 22.00	869	49.94

3.3 The confidence level of respondents in receiving wellness service

The confidence level of respondents in receiving wellness service is presented in Table 3. The survey found that during the Covid-19 pandemic, the respondents felt safe about receiving the service at a very low score level (1.28±0.58) and also desire to receive wellness service at a very low score level (1.54±0.73) while the burden of receiving service was at a very high score level (4.27±1.04). After the pandemic ended, the respondents' desire to receive wellness services at a high level (3.45±0.90) same as before the Covid-19 pandemic (3.87±0.96). The pandemic of Covid-19 had an effect on decision-making in wellness services at a very high level. In comparison, most respondents have a very low confidence level in the safety of going out to do activities during Covid-19. Respondents are concerned about the impact of coronavirus on health services and socio-economic infrastructures, personal safety, and the likelihood of voluntary self-isolation. Personal safety concerns were highest in those with negative illness attitudes and higher fight-flight freeze system (FFFS, reflecting fear/avoidance) scores (Bacon & Corr, 2020). Before Covid-19 and after Covid-19 pandemic, the respondents desired to receive wellness services at a high level. The wellness program is important in promoting people's concern about their health status, the possibility of sickness, and tailored treatment for their health (Abdullaha & Lee, 2012). So, before and after the Covid-19 pandemic, most respondents still want to receive wellness services.

Table 3 The confidence level of respondents in receiving wellness service

Decision factors	Before Covid-19		During Covid-19		After Covid-19	
	Mean ± S.D.	Importance levels	Mean ± S.D.	Importance levels	Mean ± S.D.	Importance levels
Desire to receive wellness service	3.87±0.96	High	1.54±0.73	Very low	3.45±0.90	High
Feeling safe about receiving the service	3.06±0.71	Medium	1.28±0.58	Very low	2.91±0.55	Medium
Burden of receiving service	2.51±1.08	Low	4.27±1.04	Very high	3.48±0.70	High

3.4 The marketing mix factors affecting respondents' decision on wellness services after the Covid-19 pandemic

The result of factors affecting respondents' decision on wellness services after Covid-19 pandemic was shown in Table 4. The wellness business which is certified by the Amazing Thailand Safety & Health Administration (SHA) certificate is the first important factor that they were concerned about (4.39±0.92). The business should be provided with high-quality (4.29±0.89), high-technology (4.22±0.92), and professional practitioners (4.26±0.92). The place should be clean (4.23±0.99), equipped with hygiene tools (4.22±0.96), and convenient for public transport (4.22±0.94). SHA certificate is the most factor that customers choose. SHA is a certificate that represents tourism industry entrepreneurs' readiness in improving their products, services, and sanitation measures which is the crucial factor in preventing the spread of Covid-19 by complying with the sanitation measures, which in turn can build trust and confidence in customers and service recipients of such products and service

(Tourism Authority of Thailand, 2020). High quality is also at a very high level. Product quality is the main important factor when purchasing a product that matches the consumer's intention. For example, in the Malaysian market, they intend to buy a product which recognizes as a high-brand name with good quality (Fernandopulle, 2020). The two factors with very high-level performance were professional and provided good advice. The professional showed interest in the service, the professional was polite, the service was adequate, and the professional, devoted full attention during the performance of the service. It can then be expected that the customer's trust in the professional and excellent service with dedication and respect are the requirements for customer loyalty in the establishment (Guedes & Silva, 2019). Most respondents emphasized the cleanliness and hygiene of the place and tools at a very high level. The hygienic conditions of the service environment and tools are also the top priority. Frequent and correct hand hygiene is one of the most important measures to prevent infection with Covid-19. Convenience to transportation takes the highest beta value in relation to customer satisfaction (Getachew, 2019). So, convenience factors were very important influential factors in the decision to serve. Moreover, most respondents are also interested in technology to support services. Technology became essential during the Covid-19 pandemic. During a time of isolation and social distancing, the world relied on technology to learn, live, and stay connected. Technology is best used to leverage and maintain social, physical, emotional, intellectual, and spiritual well-being (Goldschmidt, 2020).

Table 4 The marketing mix factors affecting respondents' decision on wellness services after the COVID-19 pandemic.

Factors	Mean ± S.D.	Importance levels
Products and service		
High quality	4.29±0.89	Very High
Technology to support service	4.22±0.92	Very High
Use famous products (Brand)	3.66±0.94	High
Famous places	3.63±0.95	High
Continuous improvement of service	3.57±0.89	High
Wide range of services	3.39±0.90	Medium
Price		
Cheaper more than other places	3.93±0.99	High
Suitable to service	3.78±0.97	High
Place		
Convenient for public transport	4.22±0.94	Very High
Shuttle service for customers	3.53±0.97	High
Promotion		
Giving a discount on special days	3.71±0.87	High
Communication channel (Application)	3.63±0.93	High
Marketing activities	3.43±0.87	High
People		
Professional	4.26±0.92	Very High
Provide good advice	4.24±0.90	Very High
Specialist services	4.14±1.04	High
Polite	3.72±0.87	High
Physical environment (Physical evidence)		
Cleanliness and hygiene	4.23±0.99	Very High
Hygiene tools	4.22±0.96	Very High
Safety	3.50±0.92	High
Adequacy of facilities	3.50±0.84	High
Process		
SHA certificate	4.39±0.92	Very High
The service process is clean and safe	4.23±0.98	Very High
Develop the service process	3.60±0.84	High
Easily to contacted	3.59±0.92	High
The service process is timely	3.59±0.89	High
Notify every step of the service	3.53±0.93	High

4. Conclusion

Most people receive wellness services for improving their appearance and lose weight. After the Covid-19 pandemic, most of them want to receive wellness services again. The wellness business which is certified by the Amazing Thailand Safety & Health Administration (SHA) certificate is the first important factor that they were concerned about. The business should be provided with high-quality, high-technology, and professional practitioners. The place should be clean, equipped with hygiene tools, and convenient for public transport.

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Analysis of influencing factors on the decision of Thai customers to use beauty clinics

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Abstract

Beauty and good looks are known as one of the most praised values among beauty customers. Beauty clinics are likely to promote the success of attractive looks. At the present, customer habits have changed especially the habits of Thai customers. This research aimed to investigate influencing factors that affect the consumer selection to use beauty clinics. The questionnaire was involved with demographic factors of customers including gender, age, occupation, and relationship. The customer behaviors in using the beauty clinic and the seven different marketing factors including product, price, place, promotion, people, process, and physical evidence were also included in the survey for investigation of influencing factors on the customer decision. The questionnaire was delivered via an online survey to Thai customers who have ever used the beauty clinic services (n=402) during February-May 2020. Data from the online survey were analyzed by different statistical programs. The demographic data showed that the percentage of female and male customers responded to the questionnaire were 78.9% and 21.1% while that of the customers' ages of 25-29 and 20-24 years old were 45.5% and 39.6%, respectively. Among the services in the beauty clinic, the special care of acne, scar, and freckles (41%) as well as common skin treatment (26%) were high required by the customers. The influencing factors of the customer decision to use the skincare beauty clinic were investigated. The results showed that the influencing factors ranking from high to low were personnel or staff, product, and procedures ($P<0.05$). The decision of customers to use the skincare beauty clinic did not differentiate between age and occupation factors. As different gender was a significant factor, female customers had a higher rate of the decision to use beauty clinic services than male customers ($P<0.05$).

Keywords: Beauty clinics; Customer decision; Customer decision; Marketing factors

1. Introduction

Beauties and good looks are known as one of the most praised values amongst consumers in current Thai society and will be continuing to increase their impact in the upcoming future. In the past, people who were interested in beauty and good looks were often middle-aged women. The women had high purchasing power because they had to use their appearances for their occupations (Thongplean *et al.*, 2012). Nowadays, the trend of beauty and good looks has increased among young adults, university students, and even men. Social business interactions require individuals to have presentable-looking faces and skin and to increase confidence and solid personality through their appearances. Moreover, Thai society is widely open to new innovations from foreign countries, especially Korean cultures which made them aware through popular artists who used the image of the beauty of their looks and skin as the selling point.

Consumer habits have changed at present, especially the habits of young consumers. These customers who have ages between fifteen years and over have increasingly used the services of beauty clinics (Fugate et al., 2008). The facial treatment of young customers includes acne treatment while in adults the treatments were for wrinkles, blemishes, and black spots. Thus, having a clear, smooth face is in high demand among today's consumers.

Recently, many beauty clinics have provided many services in Bangkok and other provinces in order to spread to various levels of consumer groups widely. Consumers also have a high chance to receive beauty services easily and get more choices that they prefer. Thus, in order to succeed in beauty clinic services, businesses must have various competitive strategies whether it is developing their products or services. The reason leads to the advancement of modern medical technology including new innovations in skin care and helping to solve various skin problems such as wrinkles, scars, acne, and freckles. The technology combines with the experience and expertise of qualified medical personnel. Expanding the franchise to make the services more convenient and also different in marketing strategies to attract the interest of consumers such as advertising (Lovelock & Wright, 2002). These concepts result in the business being able to retain sustainable growth.

Therefore, the study aimed to study the different factors that affect customers' decisions to use beauty clinics. The questionnaire including the demographic characteristics and marketing mix factors was used to evaluate the customers' decisions via the online survey. This approach comes with the hope that the information will be useful to be applied as a guideline to improve the services of the beauty clinics in order to be able to respond to the customers' needs appropriately and efficiently.

2. Materials and Methods

2.1 Sample population

The sample population of this research were consumers who ever used the skincare beauty clinic services throughout the country by using Google Docs Form via social networks.

2.2 Determination of sample size

Since the researchers could be not aware of the exact population that uses the skincare beauty clinic services throughout the whole country. The researchers decided to use the formula calculating the sample size used to estimate the proportion of the population by specifying the highest variance level which is p = 0.5 and q = 0.5 by accepting the margin error of 5% at a 95% confidence level and the Cochran formula to estimate the sample (Cochran, 1963) therefore:

$$n = \frac{Z^2pq}{E^2}$$

by
n = sample size
z = confidence level
p = the likelihood of an event or the proportion of the population of the characteristics of interest in the sample (p)
q = chance of an event not happening equal to 1 - p in the case of the sample
e = sampling tolerances

Substitute

$$n = \frac{(1.96)^2(0.5)(1-0.5)}{(0.5)^2}$$

$$= 384.16$$

According to the above sample size calculation the population equaled to 385 people. Nonetheless, researchers specified the sample size in this research increase by 4% from the total sample size which was calculated at 400 people increase from 385 in order to prevent mistakes from incorrectly answered questionnaires by using purposive sampling which is the group of people who use beauty clinics.

2.3 Factors used in the research

2.3.1 Marketing mix factors (7P's)

The marketing mix factors (7P's) included (1) Product, (2) Price, (3) Place, (4) Promotion, (5) People or Employee, (6) Process and (7) Physical Evidence (Leelavorapong *et al*., 2019).

2.3.2 Demographic factors

The demographic factors in this work were (1) Gender, (2) Age, and (3) Occupation.

2.3.3 Following variables

This was the decision to use the skincare beauty clinic.

2.4 Research procedure

This research was quantitative research that uses a questionnaire as a tool to collect data.

The questionnaire was divided into three parts which are:

Part 1: Preliminary screening questions of the respondents. The questions were about their general behavior in using skincare beauty clinic services.

Part 2: Questions regarding marketing mix and the level of the consumers' decision on using skincare beauty clinics. In the evaluation, the Rating Scale tool which has the criteria for determining the weight of the assessment into 5 levels according to the Likert Scale method as follows:

Comments Level	Rating Level
Very High	5 points
High	4 points
Moderate	3 points
Low	2 points
Very Low	1 point

For the interpretation criteria for rating the average score, the researchers applied the concept of the average interpretation using the formula for finding the width of the inter-spacing is as follows

$$\frac{Highest\ Points - Lowest\ Points}{Number\ of\ levels} = \frac{5-1}{5} = 0.80$$

4.21-5.00 means most consumers agree/use the service.
3.41-4.20 means that consumers agree with it / may use the service.
2.61-3.40 means that consumers agree moderate/uncertain.
1.81-2.60 means that consumers agree little / may not use the service.
1.00-1.80 means that consumers agree the least / definitely do not use the service.

Part 3**:** Demographic information of the respondents. This comes questionnaire form of a checklist.

Therefore, the validity and reliability of the questionnaire were corrected by checking the legitimacy of the statements and complete coverage.

2.4.1 Data collection

Data in the research were collected by using questionnaires from 400 people. In this case, the questionnaires were handled through the survey by Google Docs Form via Social Network.

2.4.2 Data analysis

After collecting all the survey answers, the data were analyzed through statistical methods as the following programs.

1) Descriptive statistics

The statistics were used for explaining the data and the nature of the data collected from the sample or population by presenting in the form of a frequency distribution table regarding percentage, ratio, average, standard deviation, and various charts.

2) Inferential statistics

The data were analyzed by using the program for statistical prefabrication to perform hypothesis testing in order to refer to all the data by using the following results analysis tools.

3) Factor analysis

The variables in each factor were evaluated in groups. These included product factors, price factors, distribution channel factors, marketing promotion factors, personnel or staff factors, process factors, and physical environmental factors.

4) Regression

This was used to study the relationship between the results from the factors being analyzed and the decision to use the beauty clinic services of the sample group

5) Chi-square

This was used to test if the demographic factor such as differences in gender, age and occupation had any effects on the decision to use the beauty clinic services of the samples, whether if it was differentiating or not.

3. Result and Discussion

3.1 Demographic characteristics

The demographic data of respondents (n=402) were collected and shown in Table 1. The result in Table 1 gender of respondents showed that 317 females (78.9%) responded to the questionnaire while males took the questionnaire 85 (21.1%). The age of respondents showed that the large groups of customers to use beauty clinic services had the ages 25-29 years old (45.5%) and 20-24 years old (39.6%). Occupation of respondents showed that the student (49.5%) and company employees (28.1%) were the most occupations to use beauty clinic services. Therefore, the result of the survey, female customers usually used the beauty clinic services more than males, and the target group that used the most services was 25-29 years old.

3.2 General behavioral data in using the skincare beauty clinic service of a sample group

The general behavioral data were collected and shown in Table 2. Information about general behaviors in using beauty clinics of the samples in this research consists of beauty clinics that were used most often, frequency of the uses of the beauty clinic services, frequency of the expenses every time

Table 1 Demographic characteristics of participants (n=402)

Characteristics	Frequency (N)	Percent (%)
Gender		
Male	85	21.1
Female	317	78.9
Age of respondents		
15-19	29	7.2
20-24	159	39.6
25-29	183	45.5
30-39	21	5.2
40 up	10	2.5
Occupation of respondents		
Government / State Enterprises	31	7.7
Company employee	113	28.1
Employee	13	3.2
Self-Employed	46	11.4
Student	199	49.5

they use the beauty clinic services, and individuals who had the most influence on the decision to use the skincare clinic services, could be seen as follows.

The top three clinics that were most used by the respondents consisted of Wuttisak cosmetics with 32%, followed by Nitipon clinic with 18 percent, and Ratchathewi clinic with 18%. Most of the respondents chose to take care of acne, scars, and freckles which was 41%, followed by skincare treatment at 26%, and skin laser which was 14%. Most of the respondents used the skincare beauty clinic 1-2 times per month 42%, followed by less than 1 time per month which was 34%, and 3-4 times per month which was 19%. The expense frequencies of the respondents when they used the skincare clinic services of the top three respondents was 500-1000 baht which was 35%, followed by 1001-2000 baht which was 34%, and 2001-3000 baht which was 16%. Individuals who had the most influence on the respondents' decision to use the skincare clinic services top 3 were themselves which was 43%, followed by their friends which was 30%, and their family which was 16%. From the previous study, Kuphadist & Yenyuak, 2020 studied about consumer behavior marketing mix factors affecting and the needs of consumers using beauty clinic services in Bangkok. The results of the study were found that among beauty program services, the most popular program was the injection program and the most frequently used clinic was Nitipon Clinic (Kuphadist & Yenyuak, 2020).

Table 2 General behavior in using the skincare beauty clinic (n=402).

General behavior	Frequency (N)	Percent (%)
Skincare clinic		
Wuttisak cosmetics	128	32
Nitipon clinic	73	18
Ratchathewi clinic	73	18
Pan clinic	49	12
Medicare clinic	34	8
Romrawin clinic	24	6
Etc.	21	6
Skincare service		
Take care of acne, scar, and freckles	165	41
Skin treatment	106	26
Skin laser	57	14
Remove birthmark	39	10
Botox	34	8
Vitamin	1	1
Frequencies of using skincare beauty clinics		
1-2 time per month	169	42
Less than 1 time per month	175	34
3-4 time per month	15	19
5-6 time per month	78	4
More than 6 times per month	5	1
Expenses value		
500-1000 Bath	140	35
1001-2000 Bath	136	34
2001-3000 Bath	63	16
3001-4000 Bath	26	7
Less than 500 Bath	25	6
More than 4001 Bath	12	3
Influencer		
Themselves	180	43
Friends	117	30
Family	63	16
Famous People	33	8
Doctor	10	3

The average score, opinion level per measure of independent variable which has influenced the decision to use the beauty clinic of the sample and variable based on data obtained from complete questionnaires that influence the decision to use the beauty clinic service is shown in Table 3.

From Table 3, it was found that the top five individuals factors that the respondents agreed on the most ranged from highest to lowest results in the equipment and tools used were safe and hit the standards (Avg 4.43), the technology used for services was up to date (Avg 4.41), the products and

Table 3 Average score and standard deviation on different factors (N=402).

Factors	Average	SD	Yield
Product factors			
This clinic is famous and reliable	4.34	.74	Very high
Variety of services	4.21	.78	High
Equipment and tools used is safe and hit the standards	4.43	.65	Very high
The technology used for services is up to date	4.41	.64	Very high
Products and services have good quality	4.37	.65	Very high
Price factors			
The price is aligning with quality of the service provided	4.30	.64	Very high
Prices are similar to clinics at the same level	4.21	.68	High
Able to pay the installments service fees appropriately	4.08	.72	High
Distribution channels			
Convenient traveling to use the services	4.21	.73	High
The number of branches of the clinic that is available	4.06	.82	High
Location that is easy to find	4.09	.78	High
Parking area	4.05	.76	High
Marketing factors			
There are promotions, discounts, giveaway packages, and interesting marketing promotions such as giving a trial use.	4.04	.74	High
Membership system/membership service	3.93	.78	High
Have presenters that are famous and interesting.	3.75	.89	High
Thorough advertisements such as TV, Internet, Booths, Public relations, etc.	3.79	.86	High
Good suggestions and relations from employees in the clinic.	3.96	.79	High
Notifying information such as new innovations letting customers aware always.	4.00	.72	High
Personnel or Employee factors			
The staffs are courteous	4.37	.67	Very high
Staffs are attentive	4.33	.68	High
The number of employees is sufficient to provide the service.	4.30	.70	Very high
Process factors			
Speed and accuracy of service	4.30	.66	Very high
Ongoing service monitoring	4.23	.63	High
The ability to provide services that suit customer need	4.21	.68	High
Specify the price clearly	4.20	.73	High
Physical environmental factors			
There are signs indicating various services, clear and easy to understand	3.96	.74	High
There are enough seats while waiting for service.	3.94	.71	High
There are various media to entertain while waiting for service.	3.85	.83	High

services had good quality (Avg 4.37), the staffs were courteous (Avg 4.37), and this clinic was famous and reliable (Avg 4.34).

Apart from this, when considering each of the main factors, the study results could be summarized as follows.

The product factors that the respondents agreed on the most were the equipment and tools used were safe and hit the standards (Avg 4.43) followed by the technology used for services being up to date (Avg 4.41).

The price factors that the respondents agreed on the most were the price aligning with the quality of the service provided (Avg 4.30: Totally agree) followed by prices similar to clinics at the same level (Avg 4.21).

The distribution channel factors that the respondents agreed on the most were convenient traveling to use the services (Avg 4.21) followed by the location that is easy to find (Avg 4.09).

The marketing factors that the respondents agreed on the most were promotions, discounts, giveaway packages, and interesting marketing promotions such as giving a trial use (Avg 4.04) followed by notifying information such as new innovations and letting customers aware always (Avg 4.00).

The personnel or employee factors that the respondents agreed on the most was the staffs are courteous (Avg 4.37) followed by staff are attentive (Avg 4.33).

The process factors that the respondents agreed on the most were speed and accuracy of service (Avg 4.30) followed by ongoing service monitoring (Avg 4.23).

The physical environmental factors that the respondents agreed with the most were the property being clean and tidy (Avg 4.19) followed by the atmosphere and beautiful place decorations (Avg 4.14).

All kinds of factors were revealed to influence the consumer's choice in choosing a beauty clinic. Therefore, the effects of respondent gender on different factors were further evaluated. The results of the gender evaluation were shown in Table 4. The results of the chi-square test showed that all factors between males and females were statistically significant ($p<0.05$). Moreover, the same factors including price, distribution channel, marketing, and process were dominated absolutely by females. From the previous study, behaviors of teenagers (19-22 years old) in Mueang Chiang Mai District in using service of beauty clinic were investigated. The result was found that most of respondents were female or 70% and the remaining 30% was male (Suriyaprom, 2009).

Table 5 Showed the summary of the relationship between age and factors that influence the consumer's choice in choosing a beauty clinic. The results of the chi-square test showed that all groups were statistically significant ($P<0.05$). Factors as follows: the clinic is famous and reliable, and the price is aligned with the quality of the service provided, convenient traveling to use the services, the number of branches of the clinic that is available, the location that is easy to find, parking area, there are promotions, discounts, giveaway packages, and interesting marketing promotions such as giving a trial use, membership system/membership service, staffs are attentive, the ability to provide services that suit customer need, specify the price clearly, there are signs indicating various services, clear and easy to understand and there are various media to entertain while waiting for service.

Table 6 showed that the factors that influence the consumer's choice in choosing a beauty clinic included the clinic is famous and reliable, variety of services, the price is aligning with quality of the service provided, the number of branches of the clinic that is available, there are promotions, discounts, giveaway packages, and interesting marketing promotions such as giving a trial use, membership system/membership service, have presenters that are famous and interesting, thorough advertisement such as TV, internet, booths, public relations etc., good suggestions and relations from employees in the clinic, notifying information such as new innovations letting customers aware always, the staffs are courteous, the number of employees is sufficient to provide the service, speed and accuracy of service, ongoing service monitoring, specify the price clearly, there are signs indicating various services, clear and easy to understand and There are enough seats while waiting for service. The factors had significant differences among the occupation ($P < 0.05$).

Table 4 Gender evaluation on different factors in using skincare beauty clinic (N=402).

Factors	Gender		P-value
	Male (N=85)	Female (N=317)	
Product factors			
This Clinic is famous and Reliable	4.18±0.74	4.27±0.74	.012*
Variety of services	4.40±0.78	4.18±0.78	.079
Equipment and tools used is safe and hit the standards	4.34±0.65	4.46±0.65	.073
The technology used for services is up to date	4.34±0.64	4.43±0.64	.030*
Products and services have good quality	4.41±0.65	4.38±0.65	.091
Price factors			
The price is aligning with the quality of the service provided	4.01±0.64	4.40±0.64	.000*
Prices are similar to clinics at the same level	4.09±0.68	4.27±0.68	.001*
Convenient traveling to use the services	4.06±0.72	4.12±0.72	.000*
Distribution channels			
Parking area	3.42±0.73	4.44±0.73	.000*
Convenient traveling to use the services	3.54±0.82	4.22±0.82	.000*
The number of branches of the clinic that is available	3.43±0.78	4.30±0.78	.000*
Location that is easy to find	3.53±0.76	4.22±0.76	.000*
Marketing factors			
There are promotions, discounts, giveaway packages, and interesting marketing promotions such as giving a trial use	3.59±0.74	4.19±0.74	.000*
Membership system/membership service	3.61±0.78	4.03±0.78	.000*
Have presenters that are famous and interesting.	3.63±0.89	3.81±0.89	.000*
Thorough advertisement such as TV, Internet, Booths, Public relations, etc.	3.67±0.86	3.84±0.86	.000*
Good suggestions and relations from employees in the clinic.	3.61±0.79	4.07±0.79	.000*
Notifying information such as new innovations letting customers aware always.	3.67±0.72	4.11±0.72	.000*
Personnel or Employee factors			
The staffs are courteous	4.26±0.67	4.42±0.67	.000*
Process factors			
Speed and accuracy of service	3.89±0.66	4.44±0.66	.000*
Ongoing service monitoring	4.00±0.63	4.31±0.63	.000*
The ability to provide services that suit customer needs	3.94±0.68	4.31±0.68	.000*
Specify the price clearly	4.18±0.73	4.26±0.73	.000*
Physical environmental factors			
There are signs indicating various services, clear and easy to understand	3.41±0.74	4.42±0.74	.000*
There are enough seats while waiting for service.	3.52±0.71	4.34±0.71	.000*
There are various media to entertain while waiting for service	4.43±0.83	3.99±0.83	.000*

Table 5 Age evaluation on different factors in using skincare beauty clinic (N=402).

Factors	Age					P-value
	15-19 (N=29)	20-24 (N=159)	25-29 (N=183)	30-39 (N=21)	40+ (N=10)	
Product factors						
This clinic is famous and reliable	4.24±0.74	4.25±0.74	4.45±0.74	4.05±0.74	4.60±0.74	.000*
Variety of services	3.80±0.78	4.16±0.78	4.26±0.78	4.33±0.78	4.70±0.78	.162
Equipment and tools used is safe and hit the standards	4.24±0.65	4.30±0.65	4.56±0.65	4.52±0.65	4.60±0.65	.135
The technology used for services is up to date	4.24±0.64	4.34±0.64	4.48±0.64	4.43±0.64	4.60±0.64	.729
Products and services have good quality	4.17±0.65	4.28±0.65	4.45±0.65	4.38±0.65	4.60±0.65	.248
Price factors						
The price is aligning with quality of the service provided	4.03±0.64	4.22±0.64	4.43±0.64	4.00±0.64	4.40±0.64	.005*
Prices are similar to clinics at the same level	4.00±0.68	4.16±0.68	4.28±0.68	4.14±0.64	4.50±0.64	.070
Convenient traveling to use the services	4.62±0.72	4.23±0.72	4.22±0.72	3.95±0.64	4.40±0.64	.000*
Distribution channels						
Convenient traveling to use the services	3.89±0.73	4.11±0.73	4.37±0.73	4.09±0.73	3.80±0.73	.001*
The number of branches of the clinic that is available	3.65±0.82	3.46±0.82	4.12±0.82	4.05±0.82	4.10±0.82	.004*
Location that is easy to find	3.65±0.78	4.03±0.78	3.39±0.78	4.05±0.78	3.90±0.78	.031*
Parking area	3.52±0.76	4.02±0.76	4.16±0.76	4.19±0.76	3.90±0.76	.000*
Marketing factors						
There are promotions, discounts, giveaway packages, and interesting marketing promotions such as giving a trial use.	3.83±0.74	4.05±0.74	4.12±0.74	3.66±0.74	3.90±0.74	.002*
Membership system / membership service	3.58±0.78	3.93±0.78	4.00±0.78	3.71±0.78	3.80±0.78	.003*

Have presenters that are famous and interesting.	3.62±0.89	3.75±0.89	3.82±0.89	3.43±0.89	3.60±0.89	.134
Thorough advertisement such as TV, Internet, Booths, Public relations, etc.	3.48±0.86	3.82±0.86	3.82±0.86	3.62±0.86	3.90±0.86	.074
Good suggestions and relations from employees in the clinic.	3.69±0.79	3.94±0.79	4.66±0.79	3.62±0.79	3.90±0.79	.098
Notifying information such as new innovations letting customers aware always.	3.69±0.72	4.02±0.72	4.05±0.72	3.76±0.72	3.90±0.72	.064
Personnel or Employee factors						
The staffs are courteous	4.52±0.67	3.90±0.67	4.39±0.67	4.14±0.67	4.20±0.67	.053
Staffs are attentive	4.34±0.68	4.28±0.68	4.37±0.68	4.47±0.68	3.90±0.68	.019*
The number of employees is sufficient to provide the service.	4.24±0.70	4.29±0.70	4.34±0.70	4.20±0.70	4.00±0.70	.085
Process factors						
Speed and accuracy of service	4.38±0.66	4.26±0.66	4.36±0.66	4.19±0.66	3.50±0.66	.072
Ongoing service monitoring	4.17±0.63	4.27±0.63	4.25±0.63	4.19±0.63	3.30±0.63	.235
The ability to provide services that suit customer needs	4.07±0.68	4.23±0.68	4.28±0.68	4.05±0.68	3.40±0.68	.030*
Specify the price clearly	4.14±0.73	4.24±0.73	4.20±0.73	4.24±0.73	3.60±0.73	.000*
Physical environmental factors						
There are signs indicating various services, clear and easy to understand	3.93±0.74	4.18±0.74	4.29±0.74	3.95±0.74	3.80±0.74	.000*
There are enough seats while waiting for service.	3.83±0.71	4.15±0.71	4.21±0.71	4.19±0.71	3.70±0.71	.201
There are various media to entertain while waiting for service.	3.62±0.83	3.95±0.83	4.01±0.83	4.05±0.83	3.80±0.83	.000*

Table 6 Occupation evaluation on different factors in using skincare beauty clinic (N=402).

Factors	Occupation					P-value
	Government (N=31)	Company employee (N=113)	Employee (N=163)	Self-employed (N=16)	Student (N=199)	
Product factors						
This Clinic is famous and Reliable	4.32±0.74	4.41±0.74	3.92±0.74	4.13±0.74	4.38±0.74	.000*
Variety of services	4.16±0.78	4.21±0.78	3.59±0.78	4.06±0.78	4.25±0.78	.043*
Equipment and tools used are safe and hit the standards	4.55±0.65	4.36±0.65	4.46±0.65	4.22±0.65	4.39±0.65	.093
The technology used for services is up to date	4.55±0.64	4.49±0.64	3.54±0.64	4.19±0.64	4.40±0.64	.126
Products and services have good quality	4.42±0.65	4.42±0.65	4.31±0.65	4.17±0.65	4.37±0.65	.443
Price factors						
The price is aligning with quality of the service provided	4.29±0.64	4.43±0.64	4.07±0.64	4.06±0.64	4.28±0.64	.036*
Prices are similar to clinics at the same level	4.03±0.68	4.23±0.68	4.31±0.68	4.22±0.64	4.22±0.64	.253
Convenient traveling to use the services	3.87±0.72	4.12±0.72	3.84±0.72	4.15±0.64	4.09±0.64	.000*
Distribution channels						
Parking area	4.39±0.73	4.24±0.73	3.92±0.73	4.04±0.73	4.22±0.73	.128
Convenient traveling to use the services	3.65±0.82	3.80±0.82	3.92±0.82	4.06±0.82	4.10±0.82	.281
The number of branches of the clinic that is available	3.93±0.78	4.06±0.78	3.85±0.78	4.13±0.78	4.07±0.78	.043*
Location that is easy to find	4.22±0.76	3.80±0.76	3.92±0.76	4.06±0.76	4.10±0.76	.087
Marketing factors						
There are promotions, discounts, giveaway packages, and interesting marketing promotions such as giving a trial use.	4.09±0.74	4.05±0.74	3.77±0.74	4.06±0.74	4.06±0.74	.027*
Membership system/membership service	3.96±0.78	4.02±0.78	3.96±0.78	3.80±0.78	4.14±0.78	.009*
Have presenters that are famous and interesting.	3.74±0.89	3.89±0.89	4.07±0.89	3.83±0.89	4.00±0.89	.000*
Thorough advertisement such as TV, Internet,	3.42±0.86	3.69±0.86	3.92±0.86	3.69±0.86	3.84±0.86	.017*

Booths, Public relations, etc.						
Good suggestions and relations from employees in the clinic.	3.39±0.79	3.76±0.79	4.00±0.79	3.61±0.79	3.89±0.79	.026*
Notifying information such as new innovations letting customers aware always.	3.80±0.72	3.96±0.72	3.77±0.72	3.76±0.72	4.01±0.72	.003*
Personnel or Employee factors						
The staffs are courteous	3.81±0.67	3.98±0.67	3.92±0.67	3.80±0.67	4.08±0.67	.003*
Staffs are attentive	4.42±0.68	4.36±0.68	4.23±0.68	4.00±0.68	4.45±0.68	.087
The number of employees is sufficient to provide the service.	4.32±0.70	4.30±0.70	4.30±0.70	4.17±0.70	4.38±0.70	.020*
Process factors						
Speed and accuracy of service	4.29±0.66	4.32±0.66	4.07±0.66	4.04±0.66	4.36±0.66	.000*
Ongoing service monitoring	4.09±0.63	4.17±0.63	4.23±0.63	4.08±0.63	4.31±0.63	.005*
The ability to provide services that suit customer needs	4.06±0.68	4.30±0.68	4.07±0.68	3.93±0.68	4.25±0.68	.080
Specify the price clearly	3.84±0.73	4.16±0.73	4.31±0.73	4.04±0.73	4.30±0.73	.013*
Physical environmental factors						
There are signs indicating various services, clear and easy to understand	4.22±0.74	4.23±0.74	3.77±0.74	4.04±0.71	4.23±0.74	.003*
There are enough seats while waiting for service.	4.09±0.71	4.14±0.71	4.23±0.71	4.06±0.71	4.16±0.71	.002*
There are various media to entertain while waiting for service.	3.87±0.83	3.88±0.83	3.85±0.83	3.96±0.83	4.02±0.83	.053

From the previous study, consumer behavior marketing mix factors affecting and the needs of consumers (18-50 years old) using beauty clinic services in Bangkok were investigated. The overall marketing mix factor affecting decision making for using beauty clinic service was at the highest level. The product was a key factor. Moreover, the overall decision making for using beauty clinic service was at the highest level. The received complete information from beauty clinics and offered discounts and promotions, and satisfaction when using beauty clinic service were influencing factors on the customers' decision (Kuphadist & Yenyuak, 2020).

4. Conclusions

In this study, the customers' decision to use the skincare beauty clinic services were investigated via the online questionnaire. As a result of the survey, female customers usually used the beauty clinic services more than males, and the target group that used the most services was 25-29 years old. Importantly, the factors that influence the decision of customers to use the skincare beauty clinic arranged from highest to lowest were the product, personnel or employee, and procedure or process. Moreover, among the services of skincare beauty clinics, customers preferred to treat acne, scars, and freckles followed by skin treatment and skin lasers.

The relationship of different factors with the Chi-square method by considering the level of significance 0.05, in terms of gender, age, and occupation, in using the skin beauty clinic. The consumers' decision to use the beauty clinic services, in general, had a relationship with the factors in the decision to use the beauty clinic. While the genders are different, there are differences in consumers' decisions to use beauty clinic services. Females have an average buying decision than males, because women may be more concerned about skin beauty care than men, so they are more interested in choosing a beauty clinic for skin care. Furthermore, the age and occupation of consumers are related to decision-making factors choose to use beauty clinics.

However, we have suggested for further study that the additional various factors of the consumers, such as consumer income factors cultural factors, and social factors should be evaluated. Other methods, such as in-depth interviews, should be used in the collection of information in order to obtain more specific information.

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Effects of music therapy and hand massage on stress, anxiety, and relaxation levels of university students

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Abstract

Stress and anxiety among university students can be caused by many factors including academic workload, time constraints, interpersonal difficulties with faculty and peers, financial problems, and even covid-19 pandemic. Among treatments to reduce stress and anxiety as well as increase relaxation, alternative methods including music therapy and hand massage have been focused on. The purpose of this study was to investigate the effects of music therapy and hand massage on the stress, anxiety, and relaxation levels of university students. In this study, thirty participants who met the inclusion criteria: getting 14-26 PSS score and not use with any stress treatment were fifteen male and fifteen female students in Mae Fah Luang University, Chiang Rai, Thailand. The participants were randomized into the following three groups: hand massage group (N=10), music group (N=10) receiving same music at 60-80 bpm without lyrics at moderate volume via headphone, and music-combined-hand massage group (N=10) receiving same music at 60-80 bpm combine with a hand massage. The interventions were conducted in participants for 20 minutes per day and performed three sections with alternative days for a week. The Visual analog scale for stress (VAS-S), State-Trait Anxiety Inventory Scale (STAI-S), and Visual analog scale for relaxation (VAS-R) were used for data collection, and the SPSS version 21 was used for data analyses. As a result of the data analysis, the interventions of the three groups were shown statistically, significantly decreased stress and anxiety, and increased relaxation ($P<0.05$). There was no significant difference among the three groups. The results indicated that music, hand massage, and music-plus-hand massage interventions were similarly effective to decrease moderate stress and anxiety and increase relaxation for university students.

Keywords: Anxiety; Hand massage; Music therapy; Relaxation; Stress

1. Introduction

The main personal stressors among adults and Generation Z (Gen Z) who are 18-21 years old were money and work, according to the American survey in 2018. Moreover, Gen Z was reported poorest mental health among other generations. Some of them have experienced symptoms of stress (The American Psychological Association, 2018). The effects of stress were headaches that can be triggered from episodic headache to chronic headache (D'amico *et al*., 2000). Furthermore, people who have a severe level of stress may risk to suicide (Feskanich *et al.*, 2002). The Department of Mental Health, Thailand (2018) had revealed that Thai people tend to be more stressed since stress problem was the most consulted in mental health service hotline in 2017 (Sauhem & Banchonhattaki, 2020). Moreover, it had been reported that the number of 11-25 age group have been increased to call for consulting mental health issues which were stress or anxiety, psychiatric, love, depression, family problems, and had thoughts or attempted to suicide (Chupradit & Chupradit, 2022). Furthermore, stress among

university students had been found and caused by many factors including academic workload, time constraints, interpersonal difficulties with faculty and peers, financial problems (Nonis *et al*., 1998), and even covid-19 pandemic.

Typically, stress could be removed by many methods including pharmaceutical treatments and alternative therapies. The administration of benzodiazepine is one of pharmaceutical treatments that can alleviate stress in the short-term by action as the sedative. However, the drug administration has undesirable effects such as memory impairment (World Health Organization, 1996). Taking antidepressant medications (e.g. duloxetine) also have undesirable effects such as nausea and dizziness (Harmer *et al*., 2008). Among alternative therapies, cognitive behavior therapy (CBT) have been reported to prevent the risk of chronic posttraumatic stress disorder in acute stress disorder patients (Bryant *et al*., 1999). Moreover, a mindfulness meditation could reduce depression, anxiety and stress (Song & Lindquist, 2015). Thus, the evidence indicated that the alternative therapies have the potential to eliminate stress.

Massage and music therapies are alternative methods to improve relaxation. Massage and other forms of complementary and alternative medicine (CAM) modalities gained popularity. The American Massage Therapy Association defines massage as "manual soft tissue manipulation, including holding, causing movement, and/or applying pressure to the body". Massage is one of CAM which has been used for centuries around the world as a therapy to promote and improve relaxation, reduces stress and anxiety (Vickers & Zollman, 1999). Hand massage is easy, fast and convenient and can be performed to various kinds of people by any caregiver without putting on or off clothing, thus making it very cost-effective. Using 10-minute slow stroke hand massage showed statistically significant improvements on psychological indicators of relaxation (Harris & Richards, 2010). Similarly, hand massage in a 10-minute time frame led to effective stress reduction (Brand *et al.*, 2013).

Another alternative method to improve relaxation, which can be commonly used is music. Baste and Gadkari (2014) revealed that music is a simple, inexpensive and effective therapy for stress from study of stress, self-esteem and depression. Music affects the central nervous system and causes distraction from the pain, leading to a state of relaxation (Nilsson, 2009). The previous studies used several types of music to reduce stress. Using easy-listening music during colonoscopic examination for the measurement of salivary cortisol level had shown to successfully reduce stress and anxiety (Uedo *et al.*, 2004). Besides, music with 60–80 beats per minute in interventions could provide the most relaxing (Ortiz, 1997). Moradipanah *et al.* (2009) examined 20 minutes listening tape that includes 3 pieces of music with 70–80 beats in patients undergoing coronary angiography. The music listening could decrease in stress, anxiety and depression levels in the group that received music.

Although massage and music therapies have revealed the effectiveness to reduce stress and increase relaxation, the intervention by these therapies to reduce stress for university students have been rarely reported. Therefore, in this study the aim was to evaluate the effects of hand massage and music on stress reduction for university students.

2. Materials and Methods

2.1 Participants

This study was carried out in Mae Fah Luang University, Chiang Rai, Thailand. The study protocol was approved by Mae Fah Luang University Ethics Committee (Protocol No: EC19360-17). The participants were 30 university students (male 15 and female 15) aged 18-25 years. Participants who met the following inclusion criteria were: in PSS score ranging from 14-26, not in the process of treating stress from doctors, able to participate in scheduled, and able to voluntarily sign to participate in the study. Participants were excluded if they had: PSS score less than 14, open wounds and infected wounds on the hand, taken stress relieving drugs, taken sleeping pills, taken sedative pills, and been a volunteer for another study related to stress reduction. The participants (N=30) were randomized to participate in this study by the sex ratio of each group was 1:1. They were randomized into 3 equal groups consist of music group (N=10), hand massage group (N=10), and music-combined-hand massage group (N=10) as shown in Figure 1.

2.2 Protocol

Conditions of experiment

The experiment environment was shut off from the comings and goings of people and the outside sound. The temperature was 25 °C.

General procedure

The participants signed the consent forms and completed the preVAS-S, STAI-S, and VAS-R questionnaire. Subsequently, they were asked to lie on the bed, close their eyes with a blindfold, cover body with a blanket at chest level and know the treatment starting. After treatment, the participants were asked to complete post VAS-S, STAI-S, and VAS-R questionnaire. Moreover, the study was conducted in participants 3 sections with alternative days for 1 week. The satisfied questionnaire was completed in the last day after treatment.

2.2.1 Music group

The participants listened to 60-80 Bpm music without lyrics that was selected by the researchers at moderate volume in YouTube application by wearing headphones (with blocking ambient noise) for 20 minutes, while the participants were asked to close their eyes. During three days the same music was played. A researcher was in the room with the participants to control the intervention process.

2.2.2 Hand massage group

The participants were asked to lie on the bed in supine position. Hand massage procedure adapted from Essentially Holistic, 2012. Hand massage with mineral oil was performed on participants for 20 minutes (each side was conducted for 10 minutes). The procedure consists of;

1. Apply enough mineral oil on hand and forearms. Then rub the oil upwards in long motions with effleurage stroke along inside and outside of the hand and upto the elbow. Repeat each side 4 times.
2. Using both thumbs massage with circular motions on the centre line of the inner and outer arm from wrist to elbow crease. Repeat each side 4 times.
3. Using both thumbs massage with circular motions around the wrist. Repeat 4 times.
4. Using one hand to support the wrist of participant. Then use the other hand gently rotate the wrist in a clockwise and anti-clockwise direction. Repeat each rotation 4 times.
5. Using one hand to support the wrist of participant. Then use the other hand slowly push hand up and down. Repeat 4 times.
6. Using both hands to hold the hand of participants. Then use the thumbs with circular motions on the groove area between the hand bones by starting massage from the base of the finger to the wrist. Repeat each groove 4 times and massage all the groove.
7. Using one hand to support the hand and wrist of the participant. Massage each finger with circular motions and gentle pull to finish. Repeat 4 times on each finger until completely all 5 fingers.
8. Using one hand to support wrist of participant. Then use the other hand gently rotate each finger in a clockwise and anti-clockwise direction. Repeat 4 times on each finger.
9. Alternating thumbs with circle motions on the palm.
10. Bend the hand up, participant rest on elbow. Scissor like frictions performed by both thumbs. Repeat 4 times.
11. Squeezing the hand of participant.
12. Effleurage along the back of the arm upto the elbow and then perform the same on the inside hand and arm. Repeat each side 4 times. Then slide off to finish.

13. Repeat to the other hand.

2.2.3 Music-combined-hand massage group

Hand massage for participants was performed in the same procedure of the hand massage group along with listening to 60-80 Bpm music without lyrics at moderate volume by wearing headphones (with blocking ambient noise) for 20 minutes.

2.3 Outcome measurement

Questionnaire was used as a tool to study psychological effects of participants before and after treatment. The questionnaire was divided into 6 parts.

Part 1: General information of participants.

Part 2: Perceived Stress Scale (PSS). It is a classic stress assessment instrument. The questions in this scale ask about feelings and thoughts during the last month (Cohen *et al.*, 1983). It composes of 10 questions with score range from 0 to 40 with higher scores indicating higher perceived stress. The stress was classified as 3 levels: low stress (scores ranging from 0-13), moderate stress (scores ranging from 14-26) and high stress (scores ranging from 27-40).

Part 3: Stress assessment of participants before and after treatment by using visual analog scale for stress (VAS-S). VAS-S is at least as discriminating as a questionnaire when it comes to highlighting differences in stress levels between two groups (Lesage, 2012). In this study, the scales were as 0-10 with the descriptive words both ends. Indicating that the 0-3 for low stress, 4-7 for moderate stress and 8-10 for high stress.

Part 4: Anxiety assessment of participants before and after treatment by using the state component of the State-Trait Anxiety Inventory Scale (STAI-S) from Spielberger (1983). STAI-S scale can be used to determine the actual levels of anxiety intensity induced by stressful procedures. It composes of 20 questions which each question is rated on a 4-point scale (not at all, somewhat, moderately so, very much so). STAI scores are commonly classified as “no or low anxiety” (20-37), “moderate anxiety” (38-44), and “high anxiety” (45-80).

Part 5: Relaxation assessment of participants before and after treatment by using visual analog scale for relaxation (VAS-R). VAS-R is a standard measure psychological test that describes the feelings of people. The factors are answered on a 5 points that person feels at the moment with “0 = not at all” to “4 = so much”.

Part 6: Satisfaction of participants after treatment and suggestion.

The calculation of satisfaction score range

$$\text{Class interval} = \frac{\text{Maximum score} - \text{Minimum score}}{\text{Number of class}}$$

$$\text{Class interval} = \frac{5 - 1}{4}$$

$$= 1$$

From the class interval calculation, it can interpret satisfaction score into 4 levels following:

Mean value	Level of satisfaction
4.00 - 5.00	Very high
3.00 - 3.99	High
2.00 - 2.99	Moderate
1.00 - 1.99	Low

2.4 Data analysis

The demographic characteristics of the participants were compared by using ANOVA test and Chi-square test. The psychological data of participants before and after intervention within

group were compared using Paired-sample T-test. The data between 3 groups were compared using ANOVA.

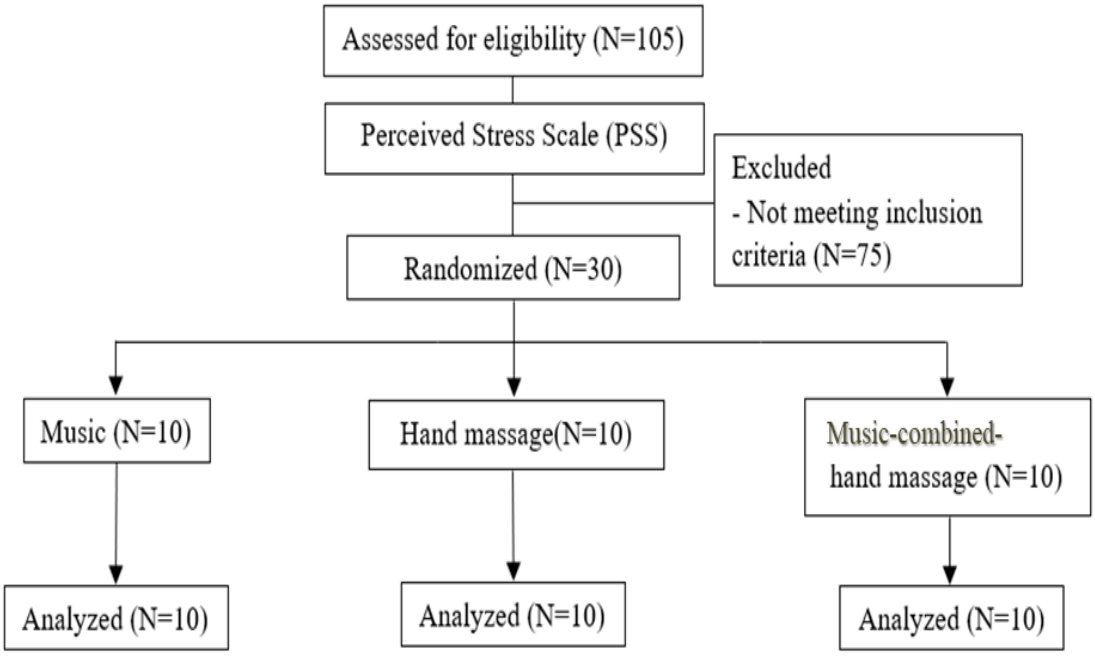

Figure 1 Flow diagram of the study

3. Results and Discussion

3.1 Perceived Stress Scale (PSS) of participants

The PSS score of 105 participants were shown in Table 1. There were 6 participants (5.8%) who had low stress level, 86 participants (83.5%) who had moderate stress level, and 11 participants (10.7%) who had high stress level. The result shown that most students in Mae Fah Luang University may have moderate stress level.

Table 1 Perceived Stress Scale (PSS) score of participants

Questionnaire	Low	Moderate	High
PSS	6 (5.8%)	86 (83.5%)	11 (10.7%)

The data were presented as N (%).

3.2 Demographic characteristics of participants (N=30)

The demographic characteristics of participants among 3 groups are shown in Table 2. The data were no statistically significant differences in age, sex, years of study, grade, income, family status, PSS score, or STAI-S score ($P>0.05$). The results among 3 groups shown that these factors were similar. Furthermore, PSS and STAI-S scores were moderate stress level in all groups.

3.3 The decreasing of stress level after intervention

The stress levels were measured by using Visual analog scale for stress (VAS-S). The results are shown in Table 3. The result was found that on day 1 the music-combined-hand massage group was the most decreased stress levels (-2.60±0.97) as compared to the music group (-1.60±1.84) and hand massage group (-1.60±1.65). On day 2 the hand massage group was the most decreased stress levels (-2.50±1.96) as compared to the music group (-1.40±1.84) and music-combined-hand massage group (-1.70±1.34). On day 3 the hand massage group was the most decreased stress levels (-1.80±1.99) as compared to the music group (-1.80±1.32) and music-combined-hand massage group (-1.60±1.08).

Table 2 Demographic characteristics of participants

Characteristics	Music	Hand massage	Music-combined-hand massage	*P-value*
Age	21.0 ± 1.16	20.8 ± 0.79	20.4 ± 0.97	0.392[a]
Sex				1.000[b]
Male	5	5	5	
Female	5	5	5	
Years of study				0.646[b]
1	1	0	2	
2	2	4	3	
3	5	4	2	
4	2	2	3	
Grade				0.714[b]
0.00 - 1.99	0	0	1	
2.00 - 2.99	6	6	5	
3.00 - 4.00	4	4	4	
Income				0.638[b]
<10,000	8	6	7	
10,000 - 19,999	2	3	3	
20,000 - 29,999	0	1	0	
Family status				0.675[b]
Live together	6	5	7	
Separated or divorced	3	3	3	
Father or mother died	1	2	0	
PSS	18.5 ± 4.50	19.9 ± 3.78	20.5 ± 3.34	0.510[a]
STAI-S	38.00 ± 10.53	42.20 ± 9.78	44.20 ± 6.97	0.324[a]

The data were presented as N except of age, PSS and STAI-S were presented as mean ± SD.
[a] mean analyzed by ANOVA test.
[b] mean analyzed by Chi-square test.

On the other hand, the results of paired *t-test* showed that stress levels in all groups were statistically significantly reduced after intervention (*P<0.05*). The previous study found that the stress in 60 females who are 20–30 years old was reduced by 10 minutes relaxation music when analyzed with the VAS (Thoma *et al.*, 2013). Furthermore, the previous study found that perceived stress in 17 women diagnosed with primary breast cancer were significantly reduce after received 30 minutes of massage (Listing *et al.*, 2010). However, our study found that the data of a one-way ANOVA test had no statistically significant difference in delta change and stress levels before and after intervention among 3 groups.

Table 3 Comparison of means score of stress levels within group and between group in each day.

Group		Day1 Mean±SD	Day2 Mean±SD	Day3 Mean±SD
Music (N=10)	Before	3.20±2.20	3.00±1.89	3.60±2.27
	After	1.60±1.51	1.60±1.27	1.80±1.55
	Change score	-1.60±1.84	-1.40±1.84	-1.80±1.32
	P-value	0.022	0.039	0.002
Hand Massage (N=10)	Before	3.80±3.36	4.30±2.36	4.20±1.93
	After	2.20±2.57	1.80±2.44	2.40±2.32
	Change score	-1.60±1.65	-2.50±1.96	-1.80±1.99
	P-value	0.013	0.003	0.019
Music-combined-hand massage (N=10)	Before	5.90±2.18	4.70±1.49	2.90±1.45
	After	3.30±1.95	3.00±1.16	1.30±1.16
	Change score	-2.60±0.97	-1.70±1.34	-1.60±1.08
	P-value	0.000	0.003	0.001
Comparing groups before intervention, ANOVA test		0.073	0.144	0.330
Comparing groups after intervention, ANOVA test		0.191	0.164	0.382
Comparing delta change, ANOVA test		0.258	0.355	0.943

The data were presented as mean±SD.

3.4 The decreasing of anxiety level after intervention

The anxiety levels were measured by using the State-Trait Anxiety Inventory Scale (STAI-S). The results are shown in Table 4. The result found that on day 1 the music-combined-hand massage group was the most decreased anxiety levels (-12.10±4.98) as compared to the hand massage group (-9.30±7.26) and music group (-7.50±6.02). On day 2 the music-combined-hand massage group was the most decreased anxiety levels (-10.20±6.71) as compared to the music group (-6.40±8.51) and hand massage group (-9.70±7.38). On day 3 the hand massage group was the most decreased anxiety levels (-11.80±8.89) as compared to the music group (-8.90±8.95) and music-combined-hand massage group (-10.30±7.39).

Table 4 Comparison of mean scores of anxiety levels within and between group in each day.

Group		**Day1 Mean±SD**	**Day2 Mean±SD**	**Day3 Mean±SD**
Music (N=10)	Before	38.00±10.53	37.80±10.68	41.60±12.66
	After	30.50±8.30	31.40±7.91	32.70±7.92
	Change score	-7.50±6.02	-6.40±8.51	-8.90±8.95
	P-value	0.003	0.041	0.012
Hand Massage (N=10)	Before	42.20±9.78	41.40±9.02	43.00±8.92
	After	32.90±8.98	31.70±5.60	31.20±5.57
	Change score	-9.30±7.26	-9.70±7.38	-11.80±8.89
	P-value	0.003	0.002	0.002
Music-combined-hand massage (N=10)	Before	44.20+6.97	43.10+7.17	39.40+7.55
	After	32.10±4.31	32.90±5.28	29.10±5.34
	Change score	-12.10±4.98	-10.20±6.71	-10.30±7.39
	P-value	0.000	0.001	0.002
Comparing groups before intervention, ANOVA test		0.324	0.422	0.720
Comparing groups after intervention, ANOVA test		0.768	0.857	0.459
Comparing delta change, ANOVA test		0.260	0.485	0.747

The data were presented as mean±SD.

On the other hand, the results of paired *t-test* showed that anxiety levels in all groups were statistically significantly reduced after intervention (*P<0.05*). Similar to previous study that used the 60-80 bpm music to reduce state anxiety by using STAI-S as an indicator. The results of the previous study found statistically significantly reduced anxiety in 180 patients who existed during the preoperative period (Uğraş *et al.*, 2018). In addition, the previous study found that STAI score after performing 10-minute hand massage was shown statistically significantly lower than the control group (Çavdar *et al.*, 2020). However, in our study result of a one-way ANOVA test found no statistically significant difference in delta change and anxiety levels before and after intervention among 3 groups.

3.5 The increasing of relaxation level after intervention

The relaxation was measured by using Visual analog scale for relaxation (VAS-R). The results were shown in Table 5. The result found that on day 1 the music-combined-hand massage group was the most increased relaxation levels (1.10±0.88) as compared to the music group (0.60±0.70) and hand massage group (0.90±0.74). On day 2 the music-combined-hand massage group was the most increased relaxation levels (1.60±0.84) as compared to the music group (0.50±0.85) and hand massage group (1.00±0.67). On day 3 the hand massage group was the most increased relaxation levels (1.40±1.08) as compared to music group (1.10±0.88) and music-combined-hand massage group (0.70±0.82).

On the other hand, the results of paired t-test showed that relaxation in all groups were statistically significantly increased after intervention (*P<0.05*). Similar to the previous study, music intervention was performed for 20 minutes per day, 3 consecutive days in 240 burn patients. The music intervention could statistically significantly increase relaxation (Ghezeljeh *et al.*, 2017). In addition, the previous study was performed by hand massage in 14 healthy women students age range 18-20 for 20 minutes. The results shown that anxiety levels were significantly decreased after hand massage (Kunikata *et al.*,

Table 5 Comparison of mean scores of relaxation levels within and between group in each day.

Group		**Day1 Mean±SD**	**Day2 Mean±SD**	**Day3 Mean±SD**
Music (N=10)	Before	3.00±0.94	2.70+0.95	2.20+1.23
	After	3.60±0.70	3.50+0.53	3.30+0.68
	Change score	0.60±0.70	0.50±0.85	1.10±0.88
	P-value	0.024	0.037	0.003
Hand Massage (N=10)	Before	2.80+0.63	2.70+0.48	2.40+0.84
	After	3.70+0.48	3.70+0.48	3.80+0.42
	Change score	0.90±0.74	1.00±0.67	1.40±1.08
	P-value	0.003	0.001	0.003
Music-combined-hand massage (N=10)	Before	2.80±1.03	2.10±0.74	3.00±0.67
	After	3.90±0.32	3.70±0.48	3.70±0.68
	Change score	1.10±0.88	1.60±0.84	0.70±0.82
	P-value	0.003	0.000	0.025
Comparing groups before intervention, ANOVA test		0.845	0.137	0.167
Comparing groups after intervention, ANOVA test		0.442	0.590	0.165
Comparing delta change, ANOVA test		0.362	0.116	0.258

2012). However, in our intervention result of a one-way ANOVA test found no statistically significant difference in delta change and relaxation before and after intervention among 3 groups.

Furthermore, the changing of stress, anxiety and relaxation in each group have similar mechanisms to affect the participant feeling. The 60-80 bpm music can gradually modify the participant's moods state. Then it induces the brain wave change to alpha wave and induce heart rate to match tempo and trigger specific neural processes (Will & Berg, 2007). Moreover, music can decrease cortisol, increase endorphin (Jiang *et al*., 2013), serotonin, dopamine (Ferreri *et al*., 2019) and oxytocin (Nilsson *et al*., 2009) that create good moods. The hand massage can stimulate parasympathetic nervous systems and decrease cortisol, increase endorphin, serotonin, dopamine, and oxytocin (Andrade, 2014) that also create good moods. Therefore, these mechanisms effect to reduce stress and anxiety and increase relaxation of participants in this study.

3.6 The satisfaction after interventions

The results of satisfaction after intervention were shown in Table 6. The satisfaction including reduced stress, reduced anxiety, more relax, stable mood and suit time showed no statistically significant difference among 3 groups. In addition, the average mean score of all groups were classified in very high level.

Table 6 Satisfaction after treatment between groups

Satisfaction	Music	Hand Massage	Music-combined-hand massage	*P-value*
Reduced stress	3.70±0.823	4.50±0.527	4.00±0.943	0.088
Reduced anxiety	4.10±1.101	4.40±0.516	3.80±0.789	0.293
More relax	4.40±0.966	4.60±0.516	4.70±0.675	0.659
Stable mood	4.00±1.333	3.70±0.675	3.70±0.823	0.737
Suit time	4.10±1.101	4.60±0.516	4.10±0.876	0.343
Average mean	4.06±1.058	4.36±0.631	4.06±0.867	

The data were presented as mean±SD.

4. Conclusions

Our results revealed that the effects of music, hand massage and music-plus-hand massage could statistically significantly decrease moderate stress and anxiety and increase relaxation for students in Mae Fah Luang University. Furthermore, there were no statistically significant differences among 3 groups of interventions, suggesting that the interventions have similar effectiveness. Therefore, these

interventions are suggested to be effective methods to relieve stress and anxiety due to their safety and low cost.

In further studies, we suggest setting up an experimental room like a spa treatment room for more comfort and the treatment time should be increased. In addition, future studies need to create a control group that does not provide any treatment and employ larger sample size for more reliable information. Furthermore, the study needs to evaluate physiological responses including pulse rate, blood pressure and cortisol levels for more accuracy of the results.

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Make-up products purchasing forecast after COVID-19 pandemic as new normal

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Abstract

This study was aimed to investigate Thai consumer's behavior and interest of make-up products as new normal after COVID-19 pandemic. The questionnaire was used for the surveillance of 624 respondents during December 2021 to March 2022. The survey found that most of the respondents were women with age ranged between 21-25 years (52.20%), students (81.90%) with income less than 15,000 baht (74.80%). Most of respondents had expense for make-up products (89.42%) and expenses per month was less than 500 baht (34.13%). The highest interested make-up was decoration on facial skin (44.98%), followed by decoration on lip (41.94%), eyebrow (9.32%) and eyelids (3.76%), respectively. Preferred channels on purchasing make-ups were both online shop and cosmetic shop (54.48%). Most of women and LGBTQ interested decorations on lip while men interested decoration on facial skin. Gender, age and occupation had an influence on purchasing's behaviors make-up. The results from this research might be used to set marketing strategic plan for make-up's products.

Keywords: Covid-19 pandemic; Facial make-up; Product demand; Purchasing behavior

1. Introduction

Thailand is one of economic area the market size of beauty and cosmetic segments have continually grown. In 2019, The revenues in beauty and personal care product in Thailand was 6.65 billion dollars and compound annual growth rate had been forecasted to growth with the rate of 5.5 percent by 2027. The largest product segment is skincare (42%), hair products (15%), soap and hygiene (14%), oral and dental care (12%), and makeup (12%) (https://www.statista.com/statistics/872695/thailand-personal-care-beauty-market-size/). Makeup products have significant effects on perceptions of facial attractiveness, competence, and socio-sexuality for both women and men place when compared with the other cosmetic segment (Aguinaldo *et al*., 2021). Covid-19 pandemic during 2020-2022 have influenced on most sectors of economy both local and global worldwide. Lockdown policy of control pandemic affects people's lifestyle that have to work from home, especially, business with contactless. Global economic recession including beauty industry caused the reduction of make-up products purchasing circulation (Gerstell *et al*., 2020). Makeup product is the most segment in cosmetics have been affected due to the pandemic causes change in consumer's behavior during quarantine at home and most of women tend not to wear make-up when stay at home. It has been revealed that circulation of make-up under mask are decreased which in contrast with make-up in unmask areas (Choi *et al*., 2022).

Online-based commerce is now popular due to the fact that the consumers' behavior has changed according to there are high competition among online platforms to recruit are large number of shops providing various alternative choices for consumer. In addition, logistic systems have now been developed to be more effective from lower cost according to management system. These factors have caused an increasing in purchasing decision using online channel including cosmetics as well as makeup

products. In 2019, consumer's purchasing behavior on the most favorite make-up products via online platform is lipsticks (Phaomanacharoen, 2019). At the present, working life style during Covid-19 pandemic become new normal that both work from home through online meeting along with work at office. Even though wearing mask still be normal policy, return to on-site working place or other activities which have to participate with many people. Makeup products would turn to become important for self-confidence. As life style as well as image perception accompanying with purchasing power might be changed, consumer information about purchasing behavior and demand on makeup products is necessity for entrepreneur to set up marketing strategy. The present study was aimed at investigating demands of make-up products in terms of type, brand's level, purchasing channel and influencing factors among Thai consumers using online survey based on Google form.

2. Materials and Methods

2.1 Participants

The study populations were Thai people. The inclusion criteria consisted of people who use social media and agree to participate this study and sign the consent form. This study was approved by the Ethics in Human Research Committee of MFU (No. EC 20244-17), and there were 624 respondents who feedback in this study.

2.2 Survey design

The questionnaire was created by using Google form (https://forms.gle/Vmgbr8mFXXAUWc5U7) which was then distributed through online via G-mail, Facebook, Line and Instagram during December 2021 to March 2022. The questionnaire was divided into 2 parts consisting of general information including gender, age, status, occupation, income and expense during Covid-19 pandemic. The second part includes purchasing frequency, expense per time, purchasing demands, demands on type and expected expense for respondents who have expense for make-up. In addition, interest about make-up types, brand's level, purchasing channels and influencing factors on purchasing make-up were also surveyed.

2.3 Statistical analysis

The descriptive statistics analysis was used to describe general information. The results were expressed as frequency, percentage, mean and standard deviation. The relationships of demographic to interest on purchasing products make-up were analyzed by using Kruskal-Wallis test and $p<0.05$ was regarded as statistical difference.

3. Results and Discussion

3.1 Demographic characteristics

The demographic characteristics of the respondents are presented in Table 1. There were 624 respondents who completed the online questionnaire. Most of respondents were women (75.30%) followed by men (13.18 %) and LGBT (10.90 %). Respondents had age ranged between 21 to 25 years (52.20%), were single (96.50 %), and were students (81.90%). Most of them had income lower than 15,000 baht (74.80%).

3.2 Respondents' purchasing behavior and interest on make-up products

Most of respondents who had expense (N=558) for make-up products purchased make-up product 1-2 times/month (83.51%). The expense per time of respondents usually was not over 1,000 baht (85.70%). Most of them (80.80%) tend to increase their makeup purchasing, especially facial skin make-up (76.10%) followed by lip and eyelid makeups (Table 2) by that the expense rate will be in the range of 500-1,000 baht (47.20%) and up to 20% of the respondents who are going to increase makeup purchase tend to purchase makeup per time ranged 1,001-1,500 baht.

When the respondents (N = 66) who had no expense for makeup were surveyed their tendency to use makeup products, more than half interest on purchasing makeup products (53.03 %) (Table 3). The most demand on makeup product of respondents was asked for both respondents who had makeup

Table 1 Demographic characteristics.

Characteristics	Frequency (N=624)	Percentage (%)
Gender		
Men	86	13.78
Women	470	75.32
LGBTQ	68	10.90
Age (years)		
Less than 15	3	0.48
15-20	216	34.62
21-25	326	52.24
26-30	54	8.65
31-35	16	2.56
36-40	7	1.12
41-50	2	0.32
Status		
Single	602	96.47
Married	10	1.60
Divorced	-	-
Others	12	1.92
Occupation		
Students	511	81.89
Company employee	74	11.86
Self-employed	20	3.20
Government officer	8	1.28
Homemaker	1	0.16
Others	10	1.60
Income/month (baht)		
Lower than 15,000	467	74.84
15,000-20,000	63	10.10
20,001-25,000	23	3.68
25,001-30,000	23	3.68
30,001-40,000	20	3.20
40,001-50,000	10	1.60
50,001-60,000	6	0.96
Higher than 60,000	12	1.92
Expense/month (exclude make-up products) (baht)		
Lower than 5,000	399	63.94
5,000-10,000	162	25.96
10,001-15,000	28	4.49
15,001-20,000	22	3.53

Table 1 (Continued)

Characteristics	Frequency (N=624)	Percentage (%)
20,001-30,000	6	0.96
30,001-40,000	2	0.32
40,001-50,000	2	0.32
Higher than 50,000	3	0.48
Expense/month (make-up products) (baht)		
No expense	66	10.58
Less than 500	213	34.13
500-1,000	209	33.50
1,001-1,500	70	11.22
1,501-2,000	32	5.13
2,001-3,000	14	2.24
3,001-3,500	5	0.80
More than 3,500	15	2.40

expense who had makeup expense (R1 respondents) and had no makeup expense (R2 respondents). The most demanded makeup products were makeup product for decoration on facial skin which was 44.98 and 57.14 % for R1 respondents and R2 respondents, respectively. Foundation was the most interesting makeup among all respondents (R1 respondents, 58.96 %; R2 respondents, 65.00 %) followed by press powder (R1 respondents, 58.96 %; R2 respondents, 46.22 %) and concealer (R1 respondents, 37.85 %; R2 respondents, 45.00 %). The other makeup product respondents demanded to purchase was lip product (R1 respondents, 41.94 %; R2 respondents, 37.14 %) especially lipstick (R1 respondents, 75.21 %; R2 respondents, 53.85 %) followed by lip tint (R1 respondents, 52.99 %; R2 respondents, 46.15 %). The R1 and R2 respondents preferred mass product which were 65.23% and 62.86%, respectively. All respondents used both online plate form and onsite cosmetic shop to purchase makeup products (estimated for 54 %). The Shopee platform was the most popular to purchase make-up for R1 respondents (74.15%) while R2 respondents used both Shopee and Watson online platforms (42.86 % and 45.71 %, respectively). Department store (R1 respondents, 87.65 %; R2 respondents, 93.10 %) as well as cosmetic shop (R1 respondents, 52.49 %; R2 respondents, 60.00 %) were major channel for makeup purchasing onsite. The most factor influencing on R1 respondents was beauty bloggers and influencers (70.60%) while R2 respondents were influenced by many factors which depend on respondents including influencers (48.57 %), the necessity in daily life (45.71 %), discount (51.43 %), etc.

3.3 Influence of respondents' demographic characteristics on purchasing behavior and interest of make-up products

The Influence of respondents' demographic characteristics of the respondents who had makeup expense (R1 respondents) including gender, age, status, occupation and income on purchase and interest of facial make-up product were determined by Kruskal-Wallis test as shown in Table 4. As can be seen in the results, gender influenced on interest in purchasing make-up, as well as makeup's brand level and purchasing channels. In the other hand, gender, age, status, occupation, and income influence on many factors on the respondents' interested in makeup as well as their behaviors in purchasing makeup by that the gender was the most factors followed by age, occupation, income, and status. As the gender was the most influencing factor on makeup purchasing decision, this factor was then analyzed for which gender should be focused their purchasing's behavior, especially the R1 respondents. All possible influencing on makeup revenue including interest in make-up product type, expense/time, brand's level, purchasing channel, future's demand as well as expected increase in makeup expenses were analyzed to obtain necessary data for the planning of marketing strategic as the results are shown in Table 5.

Table 2 Respondents' purchasing behavior on make-up products of respondents who had make-up expenses (N=558).

Respondents' purchasing behavior	Respondents who had make-up expenses	
	Frequency (N)	Frequency (N)
Purchasing frequency		
1-2 times/month	466	83.51
3-4 times/month	70	12.55
> 4 times/month	22	3.94
Make-up expense/time (baht)		
< 500	241	43.19
500-1,000	237	42.47
1,001-1,500	50	8.96
1,501-2,000	17	3.05
> 2,000	13	2.33
Make-up expense/time (baht)		
< 500	241	43.19
500-1,000	237	42.47
1,001-1,500	50	8.96
1,501-2,000	17	3.05
> 2,000	13	2.33
Predication demand on purchasing make-up in future		
Increase	451	80.82
No increase	107	19.18
Demand on make-up type in future*		
Decoration on facial skin	343	76.05
Decoration on lip	205	45.45
Decoration on eyelid	190	42.13
Decoration on eyebrows	117	25.94
Expected expense is increased for purchasing make-up (baht)		
< 500	77	17.17
500-1,000	213	47.23
1,001-1,500	84	18.62
1,501-2,000	33	7.32
> 2,000	44	9.76

Even though age also influence on make-up interest, most of respondents were students which are similar to focus group, therefore, age could not be classified difference in this study.

The women and LGBT respondents were interested in facial skin makeup and lipstick while men were mainly interested in facial skin makeup. Lipsticks are important component for women in their daily grooming ritual and is considered by many as necessary addition to their face in order to feel presentable, comfortable and more confident. Considering the wide acceptance by society of lipstick as

Table 3 Respondents' interest on make-up products.

Respondents' interest in makeup products	Respondents who had make-up expenses		Respondents who had no make-up expenses	
	Frequency (N)	Percentage (%)	Frequency (N)	Percentage (%)
No interest make-up			31	46.97
Interest make-up	558	100.00	35	53.03
Decoration on facial skin *	**251**	**44.98**	**20**	**57.14**
Foundation	148	58.96	13	65.00
Press powder	116	46.22	11	55.00
Concealer	95	37.85	9	45.00
Blusher	94	37.45	6	30.00
Make-up base	60	23.90	10	50.00
Others	25	9.96	2	10.00
Contour	21	8.37	4	20.00
Highlight	19	7.57	4	20.00
Shading	15	5.98	4	20.00
Bronzer	7	2.79	3	15.00
Decoration on eyebrows *	**52**	**9.32**	**1**	**2.86**
Eyebrow pencil	40	76.92	1	100.00
Eyebrow powder	21	40.38	-	-
Eyebrow mascara	9	17.31	-	-
Eyebrow gel	7	13.46	-	-
Others	1	1.92	-	-
Decoration on eyelid *	**21**	**3.76**	**1**	**2.86**
Eye shadow	18	85.71	1	100.00
Mascara	12	57.14	-	-
Eyeliner	12	57.14	-	-
Shimmer	5	23.81	-	-
Inner eyeliner	4	19.05	-	-
Glitter	3	14.28	-	-
Others	2	9.52	-	-
Decoration on lip *	**234**	**41.94**	**13**	**37.14**
Lipstick	176	75.21	7	53.85
Lip tint	124	52.99	6	46.15
Lip gloss	67	28.63	6	46.15
Others	20	8.55	4	30.77
Lip liner	1	0.43	-	-

Table 3 (Continued)

Respondents' interest in makeup products	Respondents who had make-up expenses		Respondents who had no make-up expenses	
	Frequency (N)	Percentage (%)	Frequency (N)	Percentage (%)
Brand's level of product which are purchasing make-up				
Mass product	364	65.23	22	62.86
Masstige product	171	30.65	12	34.28
Prestige product	23	4.12	1	2.86
Channels for purchasing make-up				
Both online shop and cosmetic shop	304	54.48	19	54.29
Online shop	137	24.55	6	17.14
Cosmetic shop	117	20.97	10	28.57
Online platform for purchasing make-up*				
Shopee	327	74.15	15	42.86
Watsons	293	66.44	16	45.71
Lazada	142	32.20	9	25.71
Konvy	117	26.53	10	28.57
Others	47	10.66	1	2.86
Cosmetic shop for purchasing make-up*				
Department store	369	87.65	27	77.14
Cosmetic shop	221	52.49	21	60.00
Convenient store	118	28.03	6	17.14
Others	5	1.19	-	-
Factors for purchasing make-up*				
Review from beauty blogger, influencer	394	70.61	17	48.57
Necessity	281	50.36	16	45.71
Discount	276	49.46	18	51.43
Promotion	232	41.58	13	37.14
Shipping free	180	32.26	12	34.28
New arrivals	62	11.11	2	5.71
Preference on presenter	33	5.91	3	8.57
Tester size of product	33	5.91	2	5.71
Cash on delivery	22	3.94	4	11.43
Limited edition	18	3.22	2	5.71
Others	6	1.08	1	2.86

*more than one answer can be chosen.

Table 4 Relationship of respondents' demographic characteristics and interest on make-up products.

Purchasing behavior and interest		Gender	Age	Status	Occupation	Income/month
R2 respondents						
Interest in make-up type	Chi-Square	70.452	2.850	2.802	5.618	6.463
	Asymp. Sig.	0.000*	0.827	0.246	0.345	0.487
Interest in make-up's brand level	Chi-Square	10.561	5.164	0.722	3.337	5.934
	Asymp. Sig.	0.005*	0.523	0.697	0.648	0.548
Purchasing channels	Chi-Square	9.899	5.253	0.688	3.444	5.704
	Asymp. Sig.	0.007*	0.512	0.709	0.632	0.575
R1 respondents						
Interest in make-up type	Chi-Square	65.560	25.506	0.162	19.071	15.081
	Asymp. Sig.	0.000*	0.000*	0.162	0.002*	0.350
Purchasing frequency	Chi-Square	42.476	2.409	2.155	5.129	6.111
	Asymp. Sig.	0.000*	0.879	0.340	0.400	0.527
Make-up expense/time	Chi-Square	14.877	25.301	3.970	26.816	50.051
	Asymp. Sig.	0.001*	0.000*	0.137	0.000*	0.000*
Interest in make-up's brand level	Chi-Square	6.033	27.845	7.666	28.915	48.427
	Asymp. Sig.	0.049*	0.000*	0.022*	0.000*	0.000*
Purchasing channels	Chi-Square	56.567	27.845	3.934	5.97	5.721
	Asymp. Sig.	0.000*	0.033*	0.140	0.309	0.573
Prediction of demand on make-ups	Chi-Square	106.785	24.952	6.050	11.587	17.399
	Asymp. Sig.	0.000*	0.000*	0.049*	0.041*	0.015*
Expected expense is increased for purchasing make-up	Chi-Square	70.119	15.566	3.166	6.375	12.065
	Asymp. Sig.	0.000*	0.016*	0.205	0.271	0.098

R1 respondents: respondents who had makeup expense; R2 respondents: respondents who had no makeup expense.

part of the daily image, women's perception of make-up is lipsticks (Ogilvie and Kristensen-Bach, 2001). In contrast, LGBTQ and men mostly preferred to purchase foundation that have been introduced into the men's grooming category. For men, Thai teenagers and young adults are now promoted for grooming trends to be appear as age. Even though all genders usually purchased makeup with the frequency around 1-2 times per month, women and LGBT tended to have higher frequency in purchasing makeup products than men. However, it seemed like men was higher makeup expense per time than women and LQBT. These results were in accordance with makeup's brand level that women and LQBT normally purchased mass level makeup products (68.64% and 61.91%, respectively) while men preferred both mass and masstige product (41.82% and 50.91%, respectively). Consumers of fast fashion as primarily women and fast fashion have business model relies on recurrent, trend-driven, impulse buying of low-cost, mass-produced such as Nivea, Sivanna, etc (Alexa *et al.*, 2021). In contrast, men purchase masstige product which mean the mass consumption of luxury goods product such as MAC, NARS, etc (Wang *et al.*, 2022). Women and men buy luxury products for different reasons that men showed to be motivated by materialistic value, status value and conspicuous value, responding more positively to luxury brands loyalty programs than women (Vitoria *et al.*, 2019). However, there are limited for men's makeup products and brands in Thailand (Vechviboonsom, 2018).

Table 5 Relationship respondents' demographic characteristics (gender) who were most interested in facial make-ups. Followed by *Significantly difference, **Chi-Square ($p < 0.05$).

Respondents' interest in makeup products	Chi-square	Asymp. Sig.	Respondents who had make-up expenses		
			Men %	Women (%)	LGBTQ %
Interest in make-up type	65.560	0.000*			
Facial skin product			76.36	40.23	50.79
eyebrows product			3.64	11.36	0
eyelid product			3.64	4.09	1.59
Lips product			16.36	44.32	47.62
Purchasing frequency	42.476	0.000*			
1-2 times/month			89.09	82.95	82.54
3-4 times/month			9.09	12.96	12.70
> 4 times/month			1.82	4.09	4.76
Make-up expense/time (baht)	14.877	0.001*			
< 500			38.18	42.72	42.86
500-1,000			40.00	43.41	41.27
1,001-1,500			12.73	9.09	9.52
1,501-2,000			3.64	2.73	4.76
> 2,000			5.45	2.05	1.59
Interest in make-up's brand level	6.033	0.049*			
Mass product			41.82	68.64	61.91
Masstige product			50.91	27.72	33.33
Prestige product			7.27	3.64	4.76
Purchasing channels	56.567	0.000*			
Online shop			30.91	23.41	26.99
Cosmetic shop			45.45	18.64	15.87
Both online shop and cosmetic shop			23.64	57.95	57.14
Predication demand on purchasing make-up in future	106.785	0.000*			
No increase			56.36	15.00	14.29
Increase			43.64	85.00	85.71
Expected expense is increased for purchasing make-up (baht)	70.119	0.000*			
< 500			29.17	16.31	16.67
500-1,000			41.67	47.59	48.15
1,001-1,500			8.33	19.52	16.67
1,501-2,000			12.50	7.22	5.55
> 2,000			8.33	9.36	12.96

For purchasing channel, both online platform and cosmetic shop still be important channel for selling makeup products. From the results, women and LGBT planned to purchase more makeup products per

time from less than 500 baht to the range of 500-1,000 baht and possibly increase up to 1,500 baht. Online shopping is convenient approach for purchasing in comparison to traditional shopping (Vasic *et al*., 2019), however, in this survey many respondents still prefer both online and on-site purchasing channels. This might be due to the fact that product trial is necessary factor for purchasing decision. From previous survey from Garbarino and Strahilevitz (2004), most women are more displayed for shopping over the internet than men that purchase make-up via cosmetic shop. Women and LGBTQ have tendency to increase expense concern about facial attractiveness more than men which is one of the predictors of overall physical attractiveness and represent one of the primary factors influencing self-esteem, sociable, assertive, and extroverted (Korich *et al*., 2008; Korich *et al*., 2011).

4. Conclusion

The respondents purchasing behavior and their interest on make-up products were surveyed. Young adult women and LGBTQ are important key for the increment of revenue from lip products (lipstick) and skin facial product (foundation) which are mass products and suitable price range should be 500-1,000 bath. Both online shops, especially on shopee platform, and cosmetic shop (department store) were necessary selling channels. Contents from beauty bloggers also be an important influence on purchasing decision. Men cosmetics became interesting target for facial makeup as a new trend for cosmetic market in Thailand. This study demonstrates that lipsticks as well as foundation are popular until now and provides surveyed information of demands on make-up products to set marketing strategic plan for make-up products. However, it is needed more surveillance widely distributed in demography in terms of occupation and income to obtain more information for strategic marketing plan.

5. Acknowledgements

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Website design for facial skin type analysis

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Abstract

Facial skin can be divided into 4 types: dry skin, oily skin, normal skin, and combination skin. It was classified by the characteristic of the skin. The objective of this study was to create a website for the classification of facial skin types and provided suggestions for taking care of each skin type. The website name "Skin analysis" was created and distributed (https://6031703038.wixsite.com/website) in January 2021. The volunteers lived in Chiang Rai ages ranging between 18-40 years. They were asked to evaluate their skin types by using a website and divided by the result of the website into 4 groups: the normal skin group (n=30), the oily skin group (n=30), the dry skin group (n=30), and the combination skin group (n=30). APM-100 was used for skin types measurement and the data was compared with skin website analysis. There were 19 volunteers (63.33%) classified in normal skin type, 29 volunteers (96.67%) classified in oily skin type, 25 volunteers (83.33%) classified in dry skin type, and 26 volunteers (86.67%) classified in combination skin type which were compatible with the website. The website was evaluated for website satisfaction. There were 515 evaluators ages ranging between 18-55 years assessed on the website. The satisfaction on the website such as convenience and ease to use, ease and understanding of questions, the benefit of website information, matching between test results and skin type, satisfaction, and the recommendation of the website were at an excellent level. In conclusion, the skin analysis website was useful for the classification of skin types and provided skin analysis that was compatible with the skin measurement device at 82.50%.

Keywords: APM-100; Skin analysis; Skin type; Website

1. Introduction

Facial skin can be divided into 4 types: dry skin, oily skin, normal skin, and combination skin (Piccioni *et al*., 2017). Dry skin has less sebum than normal skin. It is unable to retain moisture leading to flaky, harsh, crake, dry, and possibly to irritation (Moniaga *et al*., 2019). People with dry skin will experience a tightening skin sensation with dull skin and a lack of suppleness. Oily skin is a common problem that occurs when oversized sebaceous glands produce excessive amounts of sebum giving the appearance of shiny and greasy skin (Sakuma & Maibach, 2012). People with oily skin have large, visible pores, and their skin is more prone to acne and other forms of acne. Normal skin has good oil and water balance. The area of the forehead, nose, and chin (known as the T-zone) may be a bit oily, but overall sebum and moisture are balanced and the skin is neither too oily nor too dry. Normal skin has smooth, small pores, no skin issues, and the skin is not sensitive. Combination skin is a combined more than one skin type, such as dry skin and oily skin (Indriyani & Sudarma, 2005). The facial sebum secretion was higher on T-zone than on both cheeks (Youn *et al*., 2005).

Nowadays, using facial care products is a daily routine for most people. Both different skin types including the skin condition can vary greatly during different periods of life. Many external and internal factors determine the condition of the skin. The external factors including weather, pollution, and medications, and the internal factor, such as stress and genetic factors, affect the level of oiliness sweat,

and skin's natural moisture factor. Most people were not able to classify their facial skin types which lead to the problem of selecting the right skin care products. The improper use of skin care products might be the reason for skin problems. Therefore, people should understand their facial skin types to avoid and solve the problems of their facial skin.

The APM-100 is a portable skin-measuring instrument that is based on microscope imaging methods equipped with a 30× magnification lens. The instrument looks like a phone connected with a 5-megapixel camera, Bluetooth, and WI-FI as presented in Figure 1. APM-100 is able to assess moisture, elasticity, sebum, pore, dark spot, acne, wrinkle, and skin thickness.

(A) (B)

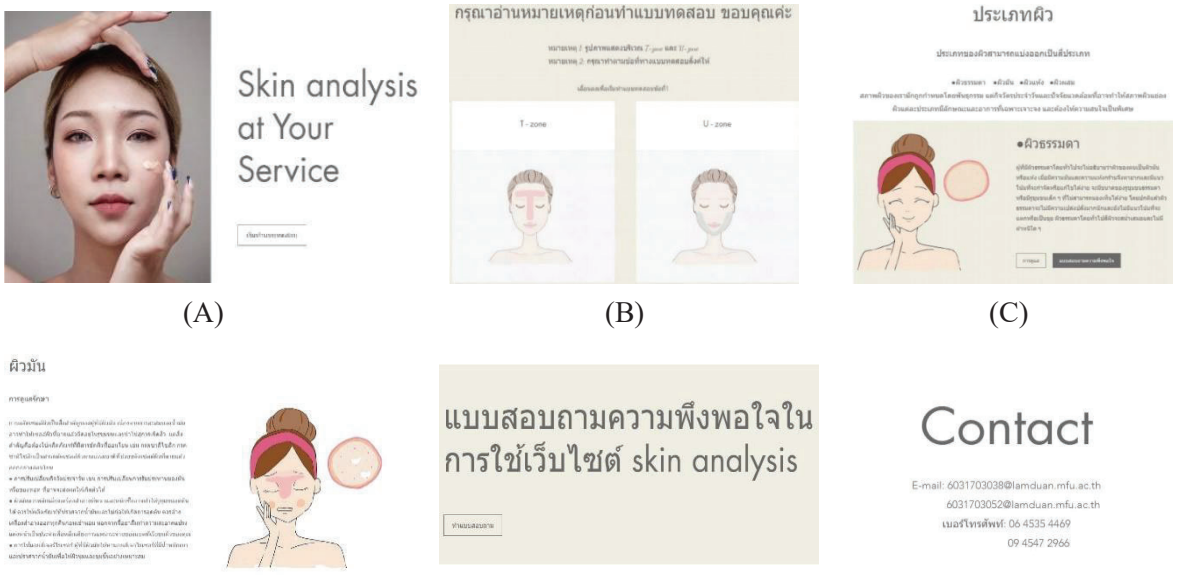
Figure 1 APM-100 measuring instrument (A) and sebum indicator patch (B)

The objective of this study was to create a website for facial skin analysis. This website was used for the classification of facial skin type and provided suggestions for taking care of each skin type. APM-100 skin measurement device was used for skin type measurement and the data was compared with skin website analysis. Skin website analysis may be helpful for those who want to know their facial skin type and skincare routine regimen.

2. Materials and Methods

2.1 Website design

The skin characteristics and various facial skin problems were used as criteria for creating a skin analysis website as shown in Table 1. The website name "Skin analysis" (https://6031703038.wixsite.com/website) was created by Wix.com and distributed online in January 2021. The website was composed of 6 windows which were; a) a home page, b) a skin analysis test, c) a skin type information, d) how to take of skin type, e) a satisfaction questionnaire, and f) a contact as shown in Figure 2.



(A) (B) (C)

(D) (E) (F)

Figure 2 Six windows in the skin analysis website

Table 1 Characteristic of each skin type

Appearance of the skin	**Skin type**			
	Normal	**Oily**	**Dry**	**Combination**
Facial skin appearance during the day				
Dry or irritate			✓	
T-zone is more oily than U-zone				✓
Oily		✓		
Dry and tight			✓	
No skin issues	✓			
Pores around cheeks				
Small	✓		✓	✓
Large		✓		
Pores around nose				
Small	✓		✓	
Large		✓		✓
Appearance of facial skin when touched				
Rough			✓	✓
Smooth	✓	✓		
The skin in the U-zone area is dry and peeling when exposed to cold temperature				
Yes			✓	✓
No	✓	✓		
Facial skin in the T-zone area is more oily than other areas after wake up				
Yes		✓		✓
No	✓		✓	
The skin is dry and tight after facial wash				
Yes			✓	
No	✓	✓		
The skin still dry and tight after moisturizer application				
Yes			✓	
No	✓	✓		
The skin is easily irritated by the weather change				
Infrequent			✓	✓
Never felt	✓	✓		
Makeup is easy to fall off during the day				
Yes		✓		✓
No	✓		✓	
Pores on the cheeks are smaller than the nose area				
Smaller				✓
Equally	✓	✓	✓	
Large		✓		
After applying skin care products, the T-zone area turns oily faster than other areas				
Yes		✓		✓
No	✓		✓	
T-zone area tend to oily than other areas				
Yes		✓		✓
No	✓		✓	

2.2 Study design

2.2.1 Comparison between skin analysis website and skin measurement device

2.2.1.1 Volunteer

One hundred and twenty healthy volunteers in Chiang Rai (aged 18-40 years) participated in this study. The volunteers were asked to evaluate their skin types by using website skin analysis followed by skin measurement with APM-100 from January to April 2021. Volunteers were divided by the result of a skin analysis website into four groups; the normal skin group (n=30), the oily skin group (n=30), the dry skin group (n=30), and the combination skin group (n=30). This study was approved by the Ethics in Human Research Committee of Mae Fah Lung University (No. EC 21012-17). All volunteers received information about the study and signed an informed consent sheet.

2.2.1.2 Facial skin preparation

Facial cleansing techniques were performed before skin measurement as following step;

Step 1. The volunteers lay on the bed in a comfortable position and covered with blankets up to chest level.

Step 2. The volunteers received facial cleansing by cleansing water, cleansing milk, and toner.

Step 3. The volunteers were rested in a room at a constant temperature of 25°C for 20 min prior to skin monitoring.

2.2.1.3 Skin measurements

The APM-100 was used for skin sebum measurement at the forehead (mid-glabella), and cheek (the most prominent area of zygoma) (Youn *et al*., 2002). The measurement area was 0.9×1.2 rectangle centimeters according to the size of the sebum indicator patch.

2.2.2 Website evaluation

Everyone who used the skin analysis website was asked to evaluate the website's satisfaction. There were 515 respondents aged 18-55 years who evaluated the website. They were asked to answer the questionnaire which consists of assessment evaluations such as convenient and easy to use, easy and understandable questions, the usefulness of website information, matching between test results and skin type, satisfaction, and recommendation of the website. The class intervals between the score were calculated as described by Okapanom & Wuttisin, (2016). Criteria for interpretation of the satisfaction level were divided into 5 levels as follows: excellent (4.21-5.00), good (3.41-4.20), moderate (2.61-3.40), fair (1.81-2.60), and poor (1.00-1.80).

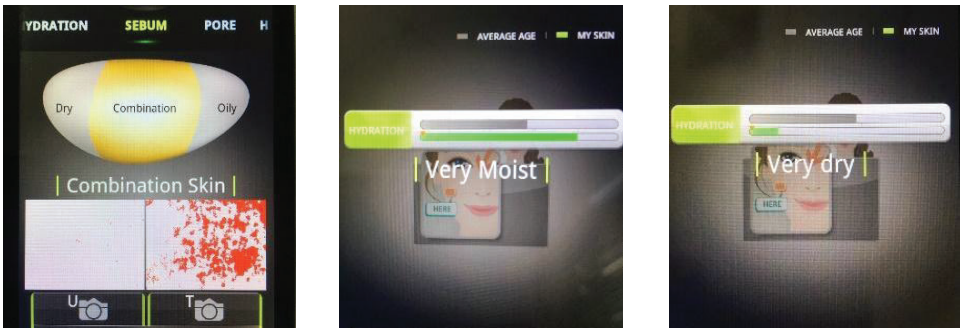
2.3 Data analysis

Descriptive statistical analyses to demonstrate the result as a percentage, arithmetic mean, and standard deviation were used for the comparisons of the result between the skin analysis website and the APM-100 measuring instrument.

3. Results and Discussion

3.1 Sebum level on sebum indicator patch

The amount of sebum collected on the sebum indicator patch at the forehead and cheek was used to classify the skin type by using APM-100. An example of skin measurement was presented in Figure 3 and Table 2. The normal skin secretes sebum in small amounts which balanced, not excessive, or lacking (Indriyani & Sudarma, 2005). Oily skin secretes a high amount of sebum level leading to shiny, greasy looks accompanied by large pores (Sakuma & Maibach, 2012). Dry skin is characterized by a scaly, rough, cracked, and fissured surface caused by a reduction in water-holding capacity (Tominaga *et al*., 2007). Combination skin has differences in sebum secretion. High sebum-secreting normally presented in T-zone (forehead, nose, and chin) while low sebum-secreting was at U-zone (both cheeks) (Youn *et al*., 2005). However, a sebum level of combination skin type on the U-zone may range from moderate, low to very low level.



(A) (B) (C)

Figure 3 Example of skin type (A), sebum level at T-zone (B) and U-zone (C) indicated by APM-100

Table 2 Example of skin type measurement by APM-100

Skin types	Sebum indicator patch	Sebum level
Normal skin type	Forehead	Low to moderate
	Cheek	Low to moderate
Oily skin type	Forehead	High
	Cheek	High
Dry skin type	Forehead	Very low
	Cheek	Very low
Combination skin type	Forehead	Moderate to high
	Cheek	Low to moderate

3.2 Comparison of skin type between skin analysis website and APM-100 measurement

The result derived from skin analysis website and APM-100 were compared to find the compatible result between website analysis and skin measurement device. From APM-100 measurement, there were 19 volunteers (63.33%) classified in normal skin type, 29 volunteers (96.67%) classified in oily skin type, 25 volunteers (83.33%) classified in dry skin type, and 26 volunteers (86.67%) classified in combination skin type which were compatible with the website as shown in Figure 4. As all of the 120 volunteers, there were 99 volunteers (82.50%) compatible with the website while 21 volunteers (17.50%) were not compatible with the website. Among 21 volunteers, 11 volunteers were classified by the website into the normal skin group but indicated into oily skin (4 volunteers), combination skin (4 volunteers), and dry skin (2 volunteers) by the APM-100 measurements. There was 1 volunteer

classified by the website into the oily skin group but indicated into dry skin by the APM-100 measurement. There were 5 volunteers classified by the website into the dry skin group but indicated into normal skin (4 volunteers) and combination skin (1 volunteer) by the APM-100 measurement. In addition, 4 volunteers were classified by a website into combination skin groups but indicated into oily skin by the APM-100 measurement. From the comparison of skin type between the skin analysis website and APM-100 measurement, the normal skin was the most incompatible with the website the reasons might be due to normal skin has various characteristics that might be the same as other skin types and the classification contents used in the website might not comprehensive enough. However, this result is based solely on APM-100 measurements. In addition to the APM-100, there are other instruments used for skin measurement by different techniques. For example, Sebumeter® is used for the classification of skin type as dry, normal, and oily based on sebum measurement (Pande & Misri, 2005). Corneometer® is used for subcutaneous moisture content measurement (Imhof & Xiao, 1998). And Tewameter® is used for the transepidermal water loss (TEWL) measurement to assess water flux out of the skin (Imhof & Xiao, 1998). These instruments were recommended for use to compare the skin type with our website.

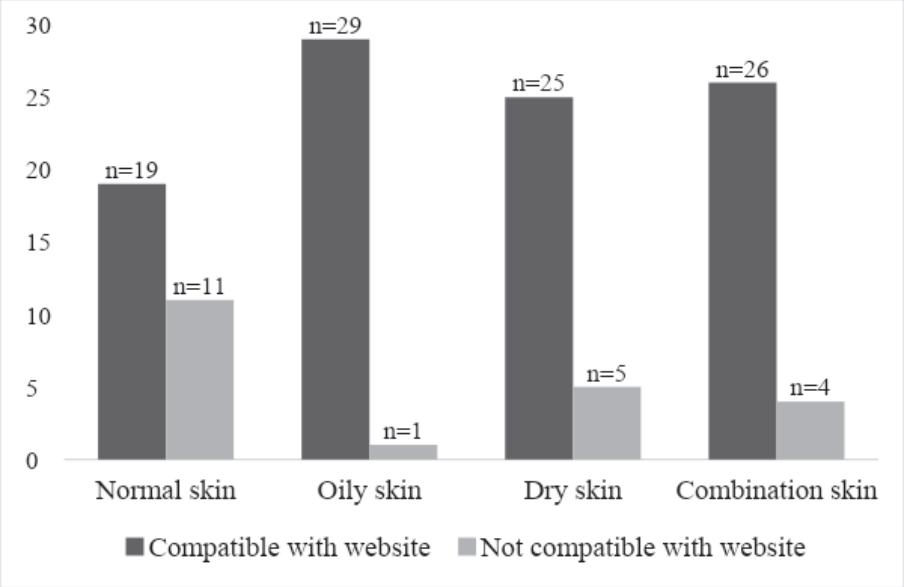


Figure 4 Comparison of skin type between skin analysis website and APM-100 measurement

3.3 Result of skin type determine from skin analysis website

There were 515 respondents who used the skin analysis website the result was presented in **Table 3**. From the website, 118 people (22.91%) classified as normal skin type, 114 people (22.14%) classified as oily skin type, 104 people (20.19%) classified as dry skin type, and 179 people (34.76%) classified as combination skin type.

Table 3 Skin type determine from skin analysis website

Skin type	Number of user	Percent
Normal skin	118	22.91%
Oily skin	114	22.14%
Dry skin	104	20.19%
Combination skin	179	34.76%

3.4 Satisfaction test

The satisfaction of the volunteers on using the skin analysis website was presented in **Table 4**. The volunteers felt that the website was convenient and easy to use, the questions were easy to understand,

the information on the website was useful, and the results of the test were consistent with their skin type. Overall satisfaction with the skin analysis website was at an excellent level.

Table 4 Satisfaction of using skin analysis website

Satisfaction	Used of skin analysis website	
	Mean±SD	Level
Convenient and easy to use	4.43±0.78	Excellent
Easy and understandable of questions	4.61±0.64	Excellent
Usefulness of website information	4.67±0.59	Excellent
Matching between test results and skin type	4.62±0.71	Excellent
Satisfaction and the recommendation of the website	4.70±0.59	Excellent

Values are expressed in Mean±SD

4. Conclusion

Skin analysis website was useful for the classification of skin types. The website provided skin analysis which is compatible with the skin measurement device at 82.50%. The website was easy to use and also provided benefits information and suggestion for taking care of each skin type properly.

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Set up a website for cosmetic products consultant and products selection

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Abstract

The objective of this study was to create a website for providing cosmetic products consultants that help consumers to choose the products. The purchasing behavior of cosmetic products was surveyed from March to April 2021. The questionnaire was constructed via Google forms and then distributed online. There were 401 respondents who completed an online questionnaire. Most people were interested in facial care products (75.06%). Then, the website for facial care products consultant and products recommendation was created by using Vue.js and the website name "V project" was distributed online (https://cosmeticprojectmfu.web.app/) for trial and asked about website satisfaction from March to April 2022. In the trial, there were 1,113 participants who responded to the questionnaire. Most of them were women (69.36%) and more than half of them were 21 to 24 years old (67.30%). The comparison of the results of each individual's skin type before and after using the website found that the results from the website are compatible with participants' thoughts (72.78%). The website was evaluated for website satisfaction. The result found that the website was convenient and easy to use with understandable questions, and the information was useful. The website was useful for the classification of skin types and provided product recommendations that are suitable for their skin type. The participants rated the website overall at an excellent level. In conclusion, the website was helping participants for choosing facial care products (97.93%) and the recommendations are suitable for their skin type (97.39%).

Keywords: Consultant; Facial care product; Online; Skin type; Website

1. Introduction

At the beginning of 2020, with the epidemic of COVID-19, most of the beauty industry was forced to shut down and sales of the beauty industry dropped dramatically. But on the other hand, the response to the COVID-19 situation in the beauty industry is positive. Because various brands changed their own manufacturing line in response to the COVID-19 situation or the production of products to prevent germs such as hand sanitizer, hand gel, and various cleaning agents. There are also promotions to increase the sales of the products as well.

The cosmetic industry is becoming very popular in the current market even though the situation of COVID-19 has not been resolved. The researcher showed that the growth of the cosmetic market will continually grow from this year until 2022 with an expected to grow of 4%, and it will grow by 5% in 2023. The cosmetic market will gradually return to growth because the behaviors of consumers see makeup as a fashion (Narla *et al*., 2021). According to Euromonitor data, it was found that the skin care group still holds the number one market share with 42%, followed by hair products (15%), body cleansing products (14%), cosmetics (12%), oral care products (12%) and deodorant (5%). When focusing on the skin care product group. It is divided into facial care products (81%), body care products (12%), sun protection products (6%), and lip products (1%) (Angus & Westbrook, 2019).

Before the COVID-19 pandemic, 70% of beauty purchases involved a visit to an offline store. During the COVID-19 pandemic, people are not comfortable going out. The purchase of products and access to services were more difficult. Many department stores were temporarily closed and people were changing their behavior to online shopping. The use of technology to facilitate the purchase of various products during the pandemic of the disease is another option (Gu *et al*., 2021).

Therefore, this research aims to survey the purchasing behavior of cosmetic products by using online questionnaires. The questionnaire asked about the types of cosmetic products that consumers are most interested in. Then, a website about the types of cosmetic products that consumers are most interested in was created. After that, the websites are distributed online for trial. The satisfaction after using the website was surveyed for website evaluation and improvement.

2. Materials and Methods

This study was divided into 3 parts; Part 1: a survey on purchasing behavior of cosmetic products in order to know the needs of consumers, Part 2: create a website for providing cosmetic products consultants to recommend the use of the product, and Part 3: a survey of consumer satisfaction from using the website.

This study was approved by the Ethics in Human Research Committee of Mae Fah Lung University (No. EC 22050- 17). All volunteers received information about the study and signed in a questionnaire for providing truthful information.

2.1 Survey on purchasing behavior of cosmetic products

The questionnaire was created by using google forms and distributed the link (https://forms.gle/L6SxUiyctkFMxP2y9) online from March 2022 to April 2022. The population of this study was Thai people who were using the internet.

The questionnaire is composed of 3 parts;

Part 1: The general information of participants such as sex, age, occupation, and salary.

Part 2: The type of cosmetic product that participants are most interested in such as hair care products, facial care products, body care products, oral care products, and deodorant products.

Part 3: The behavior of participants such as channels for purchasing cosmetic products, frequency of purchasing cosmetic products, factors affecting the purchase of cosmetic products, what channels participants receive news about cosmetic products, the price of cosmetic products, which channel participants received advice on cosmetic products and the interest in a website that helps participants buy cosmetic products.

2.2 Website design

The results from the survey on the purchasing behavior of cosmetic products showed that most people are interested in facial care products. So, we created the website by using Vue.js. Our website is named V project and distributed via the link (https://cosmeticprojectmfu.web.app/).

An example of our website was presented in Figure 1.

The website is composed of 4 parts:

Part 1: General information of participants such as name, age, and sex as shown in Figure 1(A).

Part 2: The questions to determine the skin type. The participants' skin type was determined by using a series of questions such as what do you think your skin looks like? Is your face's T zone oily? After washing your face with facial cleanser, what does your skin feel like? If you don't apply a moisturizer on your face, do you feel that your skin is dry and tight? When the air is dry, how does your skin feel when you do not apply a moisturizer? After applying a moisturizer for 2-3 hours, what does the skin on your cheeks look like? After applying a foundation without powder on your face for 2 hours, what does the skin look like? Do you have blackheads or whiteheads? The examples of questions were shown in Figure 1(B). Each question had a score. The total score was calculated and used to classify the skin type. The criteria for interpretation of the skin type were presented in Table 1.

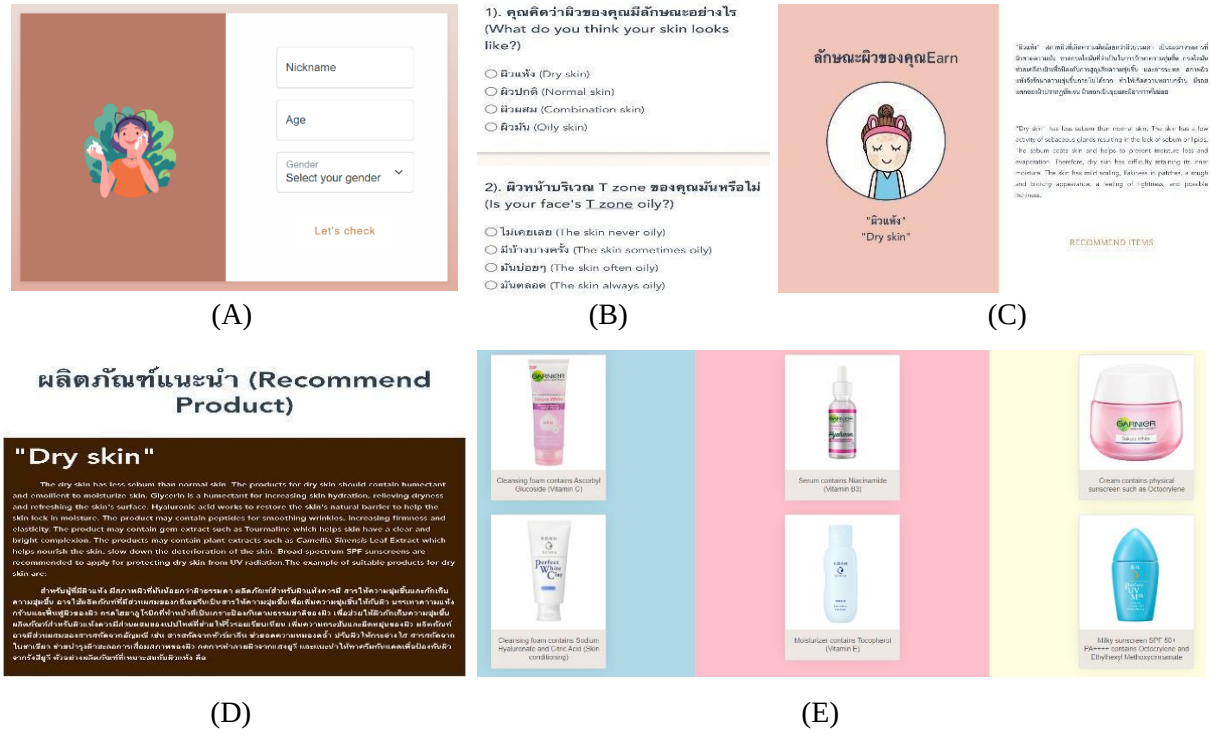
Part 3: The results of skin type from the website. Our website divided the skin into 8 types such as dry skin, dry skin and tendency to irritate, normal skin, normal skin and tendency to irritate, combination skin, combination skin and tendency to irritate, oily skin, oily skin, and tendency to irritate. The example of the result presented to participants was shown in Figure 1(C).

Part 4: The recommended product. The guideline for recommendations of suitable products for each

skin type was presented. An example of the recommended product was showed in Figure 1(D) and Figure 1(E).

Table 1 The criteria for interpretation of the skin type

Item	Score
Dry skin	8-13
Normal skin	14-23
Combination skin	24-29
Oily skin	Over 29
Non irritated	13-31
Irritated	Over 31



(A) (B) (C)

(D) (E)

Figure 1 Home page (A), a question about the skin type (B), the result of the skin type (C), skin appearance (D), product recommend (E)

2.3 A survey of user's satisfaction of websites

The satisfaction after using the website was surveyed for website evaluation and improvement. The online questionnaire (https://forms.gle/ageWQn2FjL41fha98) was used to survey the user's satisfaction with the website.

The questionnaire is composed of 4 parts:

Part 1: General information about participants such as name, age, and sex

Part 2: The questions about participants' skin type, results of the skin from the website, the information about the recommended products, and the usefulness of the website.

Part 3: The satisfaction of the website. Everyone who used the website was asked to evaluate the website's satisfaction. They were asked to answer the questionnaire which consists of assessment evaluations such as easy to use, understandable, usefulness of website information, matching between test results and skin type, satisfaction, and recommendation of the website. The class intervals between the scores were calculated as described by Okapanom & Wuttisin, (2016). Criteria for interpretation of the satisfaction level were divided into 5 levels as follows: excellent (4.21-5.00), good (3.41-4.20), moderate (2.61- 3.40), fair (1.81-2.60), and poor (1.00-1.80).

Part 4: The product recommendation section after using the website such as the recommended products are suitable for their skin type, and the recommended products helped with choosing the cosmetic product.

2.4 Data analysis

Descriptive statistical analyses were used to demonstrate the result as a percentage, arithmetic mean, and standard deviation.

3. Results and Discussion

3.1 A survey on purchasing behavior of cosmetic products in order to know the needs of consumers

The general information of the participants in the survey is shown in Table 2. There were 401 participants who responded to the survey. More than half of them were women (81.30%). Most of the participants were in the age range of 21 to 30 years old (50.12%). Most of the participants were students (86.03%) who had a salary range of fewer than 18,000 baht (85.04%).

Table 2 The general information of participants (N=401)

Item	Count	Percentage (%)
Gender		
Female	326	81.30
Male	41	10.22
LGBTQI+	34	8.48
Age (years old)		
Less than 20	164	40.90
21-30	201	50.12
31-40	25	6.23
41-50	11	2.74
Occupation		
Student	345	86.03
Office worker	43	10.72
Government officer	8	2.00
Personal business	5	1.25
Salary		
<18,000 baht	341	85.04
18,001-25,000 baht	25	6.23
25,001-32,000 baht	13	3.24
32,001-45,000 baht	8	2.00
>45,000 baht	14	3.49

Type of cosmetic product that participants are most interested in are shown in Table 3. It was found that most of them were interested in facial skin care products (75.06%). Anti-pollution and anti-acne products were the most interesting for participants (34.88%) and nowadays, younger consumers or Gen Z cite acne as their number one skin concern (Herich, 2019). The behaviors of participants in purchasing cosmetic products are shown in Table 4. It was found that participants were most likely to purchase cosmetic products from online shops (44.14%) which was the same result as the research about online shopping during the COVID-19 pandemic (Erjavec & Manfreda, 2022). They were purchased 2 to 3 times per month (36.66%). The quality of the product was the main factor in purchasing products (85.79%). Most of the participants received news about cosmetic products from Instagram (64.84%). The price that the participants were satisfied to purchase was 201 to 300 baht (33.67%). Most of the participants received advice about the cosmetic product from recommendations or product reviews (59.10%). Most of the participants were interested in media that could help them in their future purchases of cosmetic products (92.27%).

Table 3 Cosmetic products that participants are interested

Item	Count	Percentage (%)
Type of cosmetic product		
Facial	301	75.06
Body	39	9.73
Hair	38	9.48
Deodorant	16	3.99
Oral	7	1.75
Type of hair care		
Anti-hair loss	15	39.47
Conditioner/Treatment	10	26.32
Anti-hair damage	7	18.42
Shampoo	5	13.16
Hair dye	1	2.63
Type of facial care		
Anti-pollution/Anti-acne	105	34.88
Moisturizer	60	19.93
Whitening	51	16.94
Cleansing/Toner	34	11.30
Sunscreen	34	11.30
Anti-aging	17	5.65
Type of Skin care		
Lotion/serum	19	48.72
Whitening	8	20.51
Soap/Shower cream	8	20.51
Scrub/Mask	4	10.26
Type of oral care		
Toothpaste	3	42.86
Mouth deodorant	3	42.86
Mouthwash	1	14.29
Type of deodorant		
Roll-on	9	56.25
Perfume	7	43.75

Table 4 The behavior of participants

Item	Count	Percentage (%)
Channel to purchase		
Online shop	177	44.14
Department store	175	43.64
Convenience store	35	8.73
Retail store	14	3.49
Frequency of purchase		
2-3 times/month	147	36.66
1 time/month	146	36.41
1 time/2 months	60	14.96
More than 3 months/time	48	11.97
Factor to purchase*		
Quality	344	85.79
Price	273	68.08
Promotion	225	56.11
Recommendation from others	223	55.61
Convenient to purchase	106	26.43
Packaging	81	20.20

Table 4 The behavior of participants (Cont.)

Item	Count	Percentage (%)
Channel to receive news*		
Instagram	260	64.84
YouTube	238	59.35
Twitter	193	48.13
Facebook	168	41.90
Word of mouth	166	41.40
Advertisement	65	16.21
Product expo	36	8.98
Price to purchase		
<100 baht	22	5.49
101-200 baht	81	20.20
201-300 baht	135	33.67
301-400 baht	60	14.96
401-500 baht	41	10.22
>500 baht	62	15.46
Channel to received advice		
Review	237	59.10
Product label	81	20.20
Doctor	33	8.23
Seller	30	7.48
Advertisement	20	4.99
Media helps to buy cosmetic products		
Interested	370	92.27
Not interested	31	7.73

* The participants were answered more than one.

3.2 A survey of user's satisfaction of website

The satisfaction questionnaire was provided by Google Forms. The general information of the participants who used the website is shown in Table 5. There were 1,113 participants who used the website and responded to the questionnaire. Most of the participants were women (69.36%) and more than half of the participants are 21 to 24 years old (67.30%).

Table 5 General information of participants who used the website (N=1,113)

Item	Count	Percentage (%)
Gender		
Female	772	69.36
Male	258	23.18
LGBTQI+	83	7.46
Age (years old)		
20 or below	306	27.49
21-24	749	67.30
25-28	54	4.85
30 or above	4	0.36

The results of the website user's satisfaction rating are shown in Table 6. The participants rated overall the website as excellent level which is easy to use (4.96±0.20), the questions are easy and understandable (4.93±0.27), the usefulness of website information (4.94±0.28), matching between results and skin type (4.81±0.45), and satisfaction of the website (4.93±0.31). Participants answered that the recommended products on the website are suitable for their skin type (97.39%) and the recommended products helped for choosing the cosmetic product in the future (97.93%) (Table 7).

Table 6 Satisfaction of using the website

Factor	Used of skin analysis website	
	Mean±SD	**Satisfaction level**
Easy to use	4.96±0.20	Excellent
Usefulness	4.94±0.28	Excellent
Understandable of questions	4.93±0.27	Excellent
Satisfaction of the website	4.93±0.31	Excellent
Matching between results and skin type	4.81±0.45	Excellent

Table 7 Percentage of participants who rate the benefit of the website

Item	Yes	No	I'm not sure
Suitable for skin type	97.39	0.27	2.34
Help for choosing the cosmetic product	97.93	0.18	1.89

3.3 Compared the result

Skin types form the website of facial products consultant was used to compare to find the compatible result between before and after using the website. From the result before using the website, there were 312 participants (28.03%) thought that their skin classified as normal skin type, 270 participants (24.26%) classified in combination skin type, 187 participants (16.80%) classified in oily skin type, 178 participants (15.99%) classified in dry skin type, 67 participants (6.02%) classified in normal and irritated skin type, 57 participants (5.12%) classified in combination and irritated skin type, 29 participants (2.61%) classified in oily and irritated skin type, and 13 participants (1.17%) classified in dry and irritated skin type as shown in Figure 2. From the result after using the website, there were 285 participants (25.61%) classified in normal skin type, 235 participants (21.11%) classified in combination skin type, 154 participants (13.84%) classified in dry skin type, 129 participants (11.59%) classified in normal and irritated skin type, 113 participants (10.15%) classified in combination and irritated skin type, 96 participants (8.63%) classified in oily skin type, 56 participants (5.03%) classified in oily and irritated skin type, and 45 participants (4.04%) classified in dry and irritated skin type as shown in Figure 2. From the overall results, it was indicated that the results from the website are compatible with the participants' thoughts (72.78%) and not compatible (27.22%).

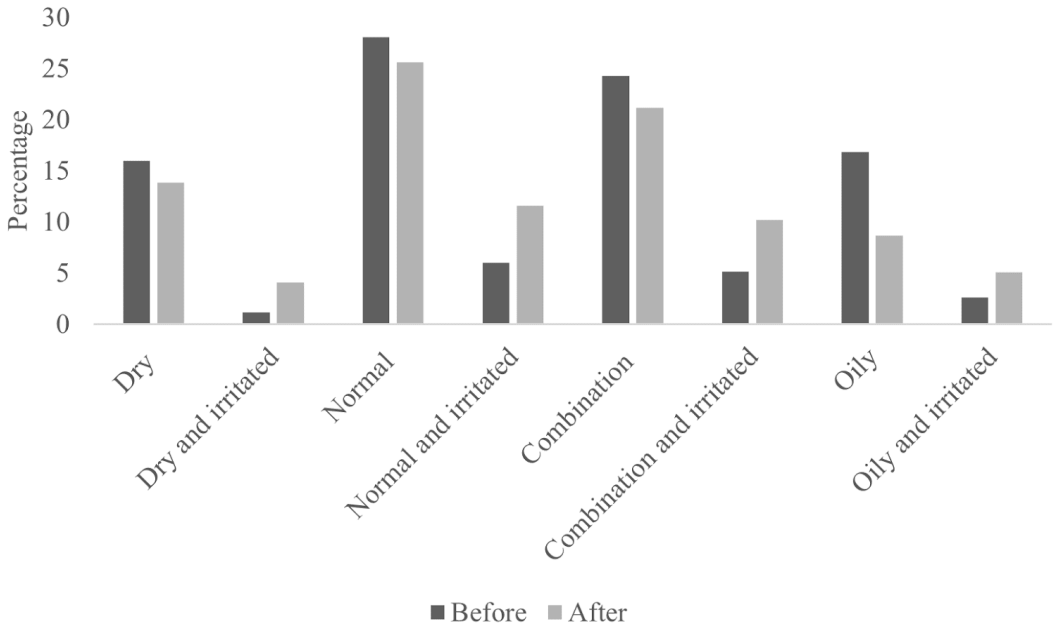


Figure 2 Comparison of the percentage of each individual's skin type before and after using the website

The website for facial care products consultants and products recommendation was created to help people by suggesting how to select suitable facial skin care products. Different skin care products need to be used based on different skin types. In order to determine the skin type, people need to have knowledge about the combination of skin conditions. Thus, this can cause difficulty for common people to analyze their skin type if they have limited skin knowledge (Noor *et al*., 2018). The skin type can be determined based on several factors such as gender, age, sleeping time, and skin conditions (Kim *et al*., 2017). As for a solution, the website was created by using a series of knowledge-based questions to determine skin type. The user answered the questions and the score was processed by the website to determine the skin type. After the skin type determination process is finished, the website provided knowledge about skin type and product recommendations with suitable chemical ingredients. However, the website still need to test for the reliability and consistency for further improvement.

4. Conclusion

Most people are interested in facial care products. Then, the website about facial care products was set up to help consumers for choosing the products that are suitable for each skin type. The participants rated the website overall at an excellent level. The website was easy to use, provided benefits information and it was helping the participants to purchase cosmetic products in the future.

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